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BOLETIM N.º 223 ZOOLOGIA N.º 21

ÍNDICE

SÃO PAULO (Brasil) — 1957

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COMPOSTO E IMPRESSO NA SECÇÃO GRÁFICA DA
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1958

SÔBRE HOLOTÚRIAS DO LITORAL SUL BRASILEIRO *

ANNA AMELIA ANCONA LOPEZ **

Com 7 Estampas

ÍNDICE

1. INTRODUÇÃO

Entre os Equinodermes da costa brasileira, os Holothurioidea compreendem uma das classes menos conhecidas taxonômicamente

Sendo animais muito freqüentes na zona entre-marés, interessantes para estudos de ecologia e de fisiologia comparada, fomos encarregados pelo Prof. Dr. Paulo Sawaya do trabalho preliminar, indispensável, de determinar o material recolhido de algumas regiões do litoral brasileiro, e especialmente do de São Paulo, a fim

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(*). Êste trabalho constitui parte da tese aprovada para obtenção do título de Doutor em Ciências, e foi realizado em parte com o auxílio da Fundação Rockefeller.

(**). Bolsista do Conselho Nacional de Pesquisas em 1952/3.

de poder efetuar os estudos fisiológicos e ecológicos sôbre alguns representantes desta classe de Equinodermes.

Iniciamos nossas pesquisas coletando material na baía de Santos e depois em São Sebastião. Mais tarde, foram-nos entregues espécimes da baía de Guanabara, do Rio de Janeiro e do Recife, Estado de Pernambuco. O material mais abundante e de maior freqüência pertence ao gênero *Holothuria*. A enumeração da maioria das espécies aqui mencionadas já foi feita em 1956 em trabalho preliminar (Ancona Lopez 1956, p. 165).

No decorrer de nossas investigações conseguimos numerosos exemplares de outros gêneros e espécies, particularmente no litoral de São Sebastião, onde se acha localizado o Laboratório de Biologia Marinha (L.B.M.) que funciona anexo ao Departamento de Fisiologia Geral e Animal. Concentramos nesse ponto os nossos estudos, dadas as facilidades excepcionais oferecidas pelo local, tendo, então, recolhido copioso material, inclusive uma espécie nova de holotúria da ordem dos *Apoda*.

Além de dar ênfase à espécie nova aqui descrita, chamaremos a atenção para a distribuição geográfica das demais espécies consideradas, incluindo algumas observações que pudemos efetuar no ambiente natural ou nos laboratórios sôbre a biologia das mesmas.

Agradecemos ao Sr. Calimério Carvalho, diretor do Aquário Municipal de Santos, pelas facilidades oferecidas para obtenção dos animais; aos funcionários do Pôsto 1 de Santos, pelo mesmo motivo; às Srtas. Alba T. Cottens e Elza Farah pelo grande auxílio na parte datilográfica e bibliográfica; ao Sr. O. G. Campiglia e seus dedicados auxiliares do Serviço de Documentação da Reitoria da Universidade de São Paulo pelo inestimável auxílio prestado na parte documentária; ao Livre-Docente Dr. Erasmo G. Mendes e ao Dr. Domingos Valente, pelos conselhos e revisão do manuscrito.

Passamos a seguir à descrição das espécies obtidas de alguns pontos do litoral brasileiro, de Cananéia ao Recife. Verificamos, desde logo, que os exemplares recolhidos pertenciam às ordens: *Aspidochirota, Dendrochirota* e *Apoda*.

Depois de apresentar a diagnose de cada espécie colhida, daremos a descrição com os pormenores tidos por importantes e, a seguir, a respectiva discussão da espécie e distribuição geográfica.

ASPIDOCHIROTA

2.

Holothuria (Holothuria) grisea Selenka 1867

1867 *Holothuria grisea* Selenka — Zeits. wiss. Zool., v. 17, p. 328, t. 17, figs. 52-56.

1882 *Holothuria grisea* Selenka, Greeff — Zool. Anz., v. 5, p. 158.

1886 *Holothuria grisea* Selenka, Théel — Chall. Exp., v. 14, p. 214.

1901 *Holothuria grisea* Selenka, Clark — Bull. U. S. Fish. Comm., v. 20, p. 258.

1907 *Holothuria grisea* Selenka, Fisher — Proc. U. S. Mus., v. 32, p. 672.

1910 *Holothuria grisea* Selenka, Sluiter — Zool. Jahrb. Syst. Suppl., v. 11, p. 333.

1928 *Holothuria atra* Jäger var. *grisea* Selenka, Panning. — Zeits. wiss. Zool., v. 132, pp. 97 e seg., Figs. 1, 2, 4, 5, 8, 9.

1929 *Holothuria* (*Halodeima*) *atra* var. *grisea*. Panning — Mitt. Zool. Statinstit. u. Zool. Museum Hamburg, v. 44, p. 49, Figs. 1-6, 9, 10, 13, 15.

1930 *Holothuria grisea* Selenka, Deichmann — Bull. Mus. Comp. Zool., v. 71, n. 3, pp. 76-77, t. 5, fig. 14.

1930 *Holothuria atra* Schmidt — Zool. Jahrb. Allg. Zool. Phys., v. 47, pp. 416, 448, 465, 466, 467, Figs. 67-68-70.

1933 *Holothuria grisea* Selenka, Clark — Echin. Pôrto Rico. v. 16, p. 10.

1935 *Holothuria* (*Holothuria*) *grisea* Selenka, Panning — Mitt. Zool. Staatinst., u. Zool. Museum Hamburg, v. 45, p. 31, Fig. 23.

1939 *Holothuria grisea* Selenka, Engel — Capita Zoologica, v. 8m pt. 4, p. 6.

1949 *Holothuria grisea* Selenka, Moussatché — Rev. Bras. Biol., v. 9, n. 4, p. 525, acetilcolina.

1951 *Holothuria grisea* Selenka, Sawaya — Ciência e Cultura, v. 3, n. 1, pp. 41-42, acetilcolina.

1951 *Holothuria grisea* Selenka, Sawaya — Ibidem, v. 6, n. 4, p. 193, acetilcolina.

1951 *Holothuria grisea* Selenka, Moussatché & Aronson — Rev. Bras. Biol., v. 11, n. 2, pp. 219-221, acetilcolina.

1953 *Holothuria grisea* Selenka, Pantin & Sawaya — Bol. Fac. Fil., Ciên. Letr. Univ. S. Paulo, Zoologia n. 18, pp. 51-59, músculos.

1953 *Holothuria grisea* Selenka, Sawaya & Mendes — Abstr. XIX Intern. Physiol. Congr., Montreal, p. 130, colinesterase.

1953 *Holothuria grisea* Selenka, Ambache & Sawaya — Physiol. comp. et Oecol., v. 3, n. 1, pp. 53-56, acetilcolina.

1955 *Holothuria grisea* Selenka, Caso — An. Inst. Biol. México, v. 26, p. 509, t. 3-4, figs. 1-10 e 1-18.

Trabalhamos com 20 exemplares (n.ºs 962-981) todos adultos, provenientes de Santos, de S. Sebastião e do Rio de Janeiro, capturados na zona da baixa-mar e conservados em formalina a 10% ou em álcool a 80%.

Os exemplares fixados em formalina eram, depois de 24 horas no máximo, transferidos para o álcool a 80%.

Para o estudo das placas calcáreas, retiramos material de diversas regiões do corpo e o tratamos com solução de hidróxido de potássio a 5%, fazendo, a seguir, a dissociação sob lupa. Conseguimos, assim, isolar as placas, montá-las entre lâmina e lamínula e desenhá-las.

No L.B.M., localizado na Praia do Segrêdo (também chamada Cabelo Gordo de Fora), mantivemos vivos diversos exemplares no aquário, durante vários dias. Pudemos, assim, medí-los em distensão máxima, chegando os espécimes nestas condições a alcançar até 40 cm de comprimento. Quando anestesiados com mentol ou com sulfato de magnésio e logo depois fixados, o comprimento reduziu-se a 25 e mesmo a 20 cm. Esta capacidade de grande contração reduzindo o comprimento do corpo é característica dêstes animais (Deichmann 1948, p. 327).

DIAGNOSE

1. Forma cilíndrica, extremidade anterior larga, parte posterior mais afilada.
2. Tentáculos: vinte, ramificados, às vêzes acastanhados, outras vêzes acinzentados como o animal, todos do mesmo tamanho.
3. Bôca circular, apresentando uma saliência, na base da qual se encontra a corôa de tentáculos.
4. Pés ambulacrais abundantes, dispostos ventralmente sem nenhuma ordenação. Ventosas pequenas.

5. Papilas dorsais menos densamente distribuídas colocadas em pequenas saliências (verrugas).
6. Anel calcáreo sem entalhes ampolares.
7. Placas calcáreas de forma variável: em forma de discos perfurados, de torres, de rosetas, de bastonetes com extremidades ramificadas.

DESCRIÇÃO

A *forma* cilíndrica. Bôca invaginada. Pés ambulacrais densamente distribuídos na face ventral. Raros na fase dorsal.

Dimensões: animais fixados, de 20 a 25 cm; animais vivos, em repouso, até 40 cm.

Côr: todos os exemplares têem coloração que vai do cinza carregado até o pardo, sendo mais escuros na face ventral.

Papilas da face dorsal providas de ventosas. Anel calcáreo formado por 10 peças apresentando um comprimento de mm 0,7. Madreporita única. Um único canal de areia, também denominado de canal pétreo.

Tentáculos, quando distendidos, atingem 30 mm e contraídos 5 mm, muito freqüentemente com coloração mais clara que o animal, mas podem mostrar-se também com a mesma coloração acinzentada.

Músculos longitudinais compostos de duas faixas ou fitas, atravessam todo o comprimento do animal, do anel calcáreo até o ânus.

Uma única vesícula de Poli, grande.

Gônada: tubos genitais formando tufo, alcançando a parte mediana do corpo e desembocando muito anteriormente na fase dorsal do animal, na altura do anel calcáreo, na superfície de um interrádio.

Tubo digestivo abrindo na parte anterior da cloaca.

Órgãos arborescentes distribuídos por todo o corpo do animal, presos por ligamentos à parede do corpo, dispostos da altura da cloaca até o 1/3 anterior do corpo.

A presença de 20 tentáculos ramificados, a distribuição dos processos ambulacrários com pés dispostos desordenadamente, a ausência de dentes anais; o anel calcáreo sem entalhes empolares; divisão muito nítida entre as partes radial e interradial, e os corpos calcá-

reos em geral formando placas como rosetas ou cavaletes, autorizanos a inclusão dos nossos espécimes no gênero *Holothuria*.

O fato de existirem: a) um só canal de areia; b) placas em forma de disco de ca. de 50μ de diâmetro (Figs. 8, 24); c) processos em forma de tôrre de ca. de 56μ de altura (Figs. 2, 3); d) rosetas com inúmeras ramificações dispondo-se sem ordem na face ventral (Figs. 4, 11); e) presença de bastonetes com extremidades ramificadas (Figs. 5, 7, 9, 12, 13, 14, 15, 16, 17, 18); f) pequenos processos achatados semelhantes a rosetas, próximos do disco terminal (Figs. 19, 21); g) existência de papilas com bastões de apôio (Fig. 23) largamente distribuídas e h) presença de placas fenetradas (Figs. 1, 6, 10, 21, 22) justificam considerar o material como pertencente à espécie *grisea*.

DISCUSSÃO

Percorrendo as publicações referentes a êste gênero, verificamos existir ainda controvérsias sôbre algumas espécies já bem conhecidas. Assim, Sven Ekman em livro recentemente publicado (1953, p. 3), ao citar a distribuição de holotúrias e suas variedades, indica como ocorrendo nas costas do Brasil a *Holothuria atra*, var. *grisea*.

Revendo a literatura, verificamos que Panning, (1931, p. 49; 1931a, p. 215) ao tratar da optica cristalina dos corpos calcáreos das holotúrias aspidoquirotas, ainda menciona *Holothuria* (*Halodeima*) *atra*, var. *grisea*, e o mesmo faz Schmidt (1930, p. 476) baseando-se no autor precedente, ao estudar os biocristais das peças esqueléticas, elementos tidos como de valor taxonômico. Todavia, o mesmo Panning em 1935 (p. 27), passa a distinguir justamente um grupo *atra* que compreende as espécies *atra, grisea, mexicana, floridana, silamensis, nitida, inornata, pulla*.

Holothuria grisea é muito semelhante à *H. atra*, mas segundo Panning (1935, p. 28), a presença de um único canal de areia em *H. grisea* distingue-a de *atra* e também de *floridana, mexicana, nitida, silamensis, inornata* e *pulla*. Já em 1931a (p. 93) o mesmo autor assinalou a diferença entre *H. grisea* e *H. atra*, caracterizada pela acumulação de rosetas e pequena ramificação das mesmas nes-

ta última espécie. Anotamos ainda que exemplares desta holotúria (*grisea*) capturada na Ilha de São Sebastião por H. Luderwaldt e W. L. Schmitt, foram classificados na Smithsonian Institution como *Holothuria grisea* (Lüderwaldt 1929, ps. 17 e 55).

Cumpre assinalar que Caso (1935, p. 511) ao descrever esta espécie indica a presença de "um dos canais pétreos mais desenvolvidos que os demais", o que faz supor que a autora admite a existência de vários canais de areia. De acôrdo com Panning (1935, p. 27), a separação de *grisea* de *atra* faz-se justamente pela presença de um único canal de areia na primeira espécie, como acontece também em todo o material por nós examinado, pois nunca distinguimos nos animais outros canais de areia. Devemos convir, porém, em que esta formação nas holotúrias, ocorrendo junto com as empolas tenactulares, se presta a confusões, sendo difícil a observação, especialmente em material fixado.

OCORRÊNCIA

Índias Ocidentais, parte oreintal; costa norte da América do Sul até o Rio de Janeiro; baía da Guiné, África Ocidental. O nosso material proveio do litoral brasileiro pesquisado até Cananéia na zona da baixa-mar até 2 m de profundidade. Panning (1935, p. 27) dá Rio de Janeiro como limite meridional desta holotúria e Deichmann (1954, p. 392) menciona sua ocorrência desde Haiti, Pôrto Rico, ao sul das Pequenas Antilhas até Rio de Janeiro. Caso (1955, p. 513) informa que o limite sul é São Paulo e Rio de Janeiro, e mais adiante diz: "Também conhecida no sul do Brasil" sem precisar o limite. Lüderwaldt (1929, p. 15) coletou exemplares desta Holotúria na Ilha de São Sebastião. De nossa parte, podemos afirmar, esta holotúria ocorre pelo menos até Cananéia, onde conseguimos identificá-la com segurança.

3.

Stichopus badionotus Selenka 1867

Figs. 25-33, 57 e 58

1867 *Stichopus badionotus* Selenka, Zeits. wiss. Zool. v. 17, p. 316, t. 18, fig. 26.

1822? *Stichopus maculatus* Greeff, Zool. Anz. v. 5, p. 158.

1883? *Stichopus assimilis* Bell, Proc. Zool. Soc. London, p. 62.
1886 *Stichopus badionotus* Selenka, Théel — Chall. Exp. v. 14, p. 196.
1888 *Stichopus xanthomela* Heilprin, Proc. Acad. Nat. Sci. Philadelphia, v. 40, p. 313 (*S. acanthomella*, Zool. Rec. 1900, p. 78 — err. tip.).
1901b *Stichopus badionotus* Selenka, Clark — Amer. Nat. v. 35, p. 494.
1916 *Stichopus badionotus* Selenka, Sluiter — Zool. Jahrb., Abt. Syst. Supl. v. II, p. 334.
1922 *Stichopus badionotus* Selenka, Clark — Bull. Mus. Comp. Zool. Harvard, v. 65, n. 3, p. 55, t. 1, 2.
1930 *Stichopus badionotus* Selenka, Deichmann — Bull. Mus. Comp. Zool., Harvard, v. 71, n. 3, p. 80, t. 5, figs. 30-36.
1933 *Stichopus badionotus* Selenka, Boone — Bull. Vanderb. Marine Museum, v. 4, p. 152, t. 98.
1939 *Stichopus badionotus* Selenka, Engel — Capita Zool. v. 8, pt. 4, p. 11.

Durante o curso de Biologia Marinha e Oceanografia Física para estudantes Latino-americanos, efetuados em São Sebastião, no L.B.M., alguns participantes, os alunos Sr. Luís Roberto Tomasi e Luís Barea, tiveram oportunidade de capturar exemplares de holotúria que logo identificamos como *Stichopus*. Em outra ocasião, obtiveram-se outros, perfazendo um total de seis, que constituiu o material de nosso estudo (n.ºs 956-961).

DIAGNOSE

1. Corpo longo, achatado, de extremidades arredondadas.
2. Tentáculos: vinte, mais claros do que o resto do corpo.
3. Na face ventral numerosos pedicelos dispostos em 3 fileiras longitudinais.
4. Na face dorsal, duas séries duplas, alternadas, de altas eminências.
5. Corpos calcáreos de formas variadas.
6. Animais fixados em álcool medem de 60 a 80 mm de largura por 210 a 300 mm de comprimento.
7. Animais vivos, em repouso, podem medir até 600 mm de comprimento.

8. Os animais conservados em álcool apresentam côr castanho-chocolate e numerosas manchas pretas em todo o corpo, bem delimitadas, e outras brancas, irregulares, mais numerosas, no dorso. Nos animais vivos as manchas são distintas, mas a coloração de fundo do corpo é róseo-alaranjada.
9. Bôca circundada por papilas.

DESCRIÇÃO

Exemplares de 70 a 80 mm de largura e 210 a 300 mm de comprimento, fixados em álcool, apresentam todos os caracteres descritos por Selenka 1867 (p. 316): os pedicelos dorsais apóiam-se sôbre altas eminências alinhadas no dorso e no limite entre o flanco e o ventre dispondo-se em duas fileiras alternadas.

A bôca colocada ventralmente é circundada por uma corôa de papilas. Os numerosos pedicelos simples do centro ordenam-se em três fileiras longitudinais, das quais as médias, duplas, são tão largas quantos as laterais. Vesícula de Poli única. Em ambos os lados do mesentério dorsal há 2 tubos com tufos de gônadas. Anel calcáreo formado por 10 peças que apresentam prolongamentos: um na parte inferior do rádio e dois na parte anterior do interradio. Corpos calcáreos em forma de tôrre (Figs. 25, 29) sendo que os das camadas subcuticulares são muito tenros, de mm 0,4 de altura; por baixo, na camada de tecido conjuntivo, há numerosos bastonetes delgados (Fig. 26) outros em forma de C, (Fig. 28), de mm 0,05-0,1 de comprimento e de bordos irregulares. Nas paredes dos pés há bastões espinhosos (Fig. 27) reticulares (Fig. 31) e processos reticulares (Figs. 30, 32, 33).

São nítidas duas séries duplas alternadas de altas eminências no dorso e na linha limítrofe com o ventre. Nesta face, os pedicelos são numerosos e colocam-se em três fileiras longitudinais, largas tanto as medianas dúplas como as laterais.

Vinte tentáculos. As placas calcáreas da tôrre apresentam uma corôa de doze dentes (Fig. 25).

DISCUSSÃO

Esta holotúria assemelha-se a *Stichopus chloronotus* Br. Segundo Selenka (l. c., p. 315), porém, as formações em tôrre dis-

tinguem *S. badionotus* de *S. chloronotus;* em *badionotus* encontra-se uma corôa com doze pontas nas torres, e em *chloronotus* a tôrre apresenta sòmente oito pontas na corôa.

A posição sistemática de *Stichopus,* como de outros gêneros de holotúrias, tem sido amplamente discutida desde que Brandt em 1835 a descreveu (Ap. Ludwig 1881, p. 591). Tratando dêste contravertido assunto, Ekman (1926, p. 435), depois de vários argumentos, conclui por deixar *Stichopus* (p. 532) na família *Holothuriidae,* subfamília *Stichopodinae*. A família, pois, compõe-se, segundo o referido autor, de três subfamílias, a saber: *Holothuriinae, Stichopodinae, Synallactinae* (p. 536), sub-famílias reestudadas por aqòuele autor e cuja classificação foi aceita por vários outros, entre êles, principalmente, Mortensen (1927, p. 358, nota do rodapé), não obstante serem consideradas por outros autores, como um grupo artificial (Perrier 1902, p. 295).

OCORRÊNCIA

Ilha de São Tomé (Guiné, África Oriental); Angola; Flórida; Praia do Segrêdo (Cabelo Gordo de Fora) em frente ao L.B.M. e ao redor do Farol do Moleque, 23° e 50' distante cêrca de 60 milhas do pôrto de Santos situado a SW. Capturada a 2-3 m de profundidade.

DENDROCHIROTA

4.

Thyone (Sclerodactyla) braziliensis Verrill 1867*

Figs. 34-44

1867 *Thyone* (*Sclerodactyla*) *braziliensis* Verrill — Trans. Acad. vol. 1, p. 370, t. 4, fig. 8a.

1897 *Thyone* (*Sclerodactyla*) *braziliensis* Verrill, Rathbun — Trans. Conn. Acad., v. 5, p. 141.

1886 *Thyone suspecta* Ludwig, Théel — Chall. Exp. v. 14, p. 133.

1955 *Thyone Naidae,* Ancona Lopez e Sawaya — Ciência e Cultura, v. 7, n. 3, p. 167.

(*) A grafia original de Verrill é *Thyone* (*Sclerodactyla*) *Braziliensis.*

Durante a estada em Recife, em 1951, o Prof. Dr. Paulo Sawaya capturou uma série de holotúrias portadoras de *Carapus* (comumente conhecidos por *Fierasfer*), peixe que foi objeto de estudo especial (Ancona Lopez 1956, pp. 389-398).

DIAGNOSE

1. Forma de fuso, oval quando contraída.
2. Tentáculos 10, sendo os 2 ventrais menores.
3. Pele fina.
4. Côr cinzento-esbranquiçada.
5. Pedicelos pequenos, numerosos, irregularmente dispostos.
6. Anel calcáreo formado por 10 peças simples com prolongamentos posteriores.
7. Placas calcáreas de várias formas, distribuídas segundo as regiões.

DESCRIÇÃO

Foram coletados 10 exemplares (n.ºs 946-955) na Praia da Piedade, em Recife.

Segundo Deichmann (1938, pp. 102-108; 1941, pp. 102; 1948, p. 354), êste gênero compreende formas de tamanho pequeno e médio, raramente de mais de 10 cm de comprimento. O corpo de pele delgada, com numerosos pés delicados, mais abundantes ventralmente. Anel calcáreo com longos prolongamentos posteriores nos radios.

A forma é oval quando contraída. Os animais são fusiformes, com 3 a 5 cm de comprimento, côr cinzento esbranquiçada, pele fina, pedicelos pequenos, numerosos, irregularmente distribuídos; dez tentáculos, sendo os dois ventrais menores. O anel calcáreo (Fig. 38) é formado por dez peças simples com prolongamentos posteriores.

Anus com dentes formados por placas calcáreas (Fig. 39), superpostas apresentando muitos orifícios. Anel oral (Fig. 38) e tentáculos com depósitos calcáreos abundantes. Vesícula de Poli e canal de areia únicos.

Espículos pequenos com placas terminais (Fig. 40) e numerosas placas de suporte, curvas (Figs. 34,35), usualmente com uma

espira bem desenvolvida, que em certas formas, contudo, está completamente reduzida. Tentáculos com delicados bastões (Fig. 36) e, às vêzes, rosetas ou taças (Fig. 37). Espículos gradualmente reduzidos com a idade em certas espécies.

Distribuição das placas calcáreas:

Tentáculos — placas muito abundantes, formando tubos compactos, placas, bastonetes e taças (Figs. 37, 41, 42) em grande número. Não há placas terminais em forma de disco.

Face ventral — placas (Fig. 43) formando o esqueleto dos pedicelos. Discos terminais com muitos orifícios. Nas paredes do corpo encontram-se taças (Figs. 37, 42).

DISCUSSÃO

O gênero *Thyone* compreende até agora 54 espécies. Do litoral brasileiro conhecem-se *Thyone* (*Sclerodactyla*) *braziliensis* Verril, assinalada nos recifes de Abrolhos, *T. cognita* (Lampert) de Fernando de Noronha, *T. suspecta* Ludwig, sem indicação precisa, *T. pervicax* Théel, da Bahia, *T. Belli* Ludwig dos recifes dos Abrolhos, Bahia (cf. Deichmann 1931, pp. 165-179).

Das espécies de Thyone do litoral brasileiro a *T.* (*esclerodactyla*) *braziliensis* é a mais discutida. Foi mencionada por Rathbun (1879, p. 241) na lista dos Equinodermes do Brasil. Por seu lado, Théel (1886, p. 133) ao tratar de *T. suspecta* Ludwig 1875, diz que o autor supõe ser esta espécie idêntica à *T. braziliensis* de Verrill, mas que as descrições de Verrill são corretas, e parece mais admissível ser distinta a espécie de Ludwig, e *T. braziliensis* provável sinônimo de *T. briareus*. O mesmo Théel (l. c.) ao discutir esta última espécie, dá-lhe *Thyone* (*Sclerodactyla*) *braziliensis* Verrill, 1867-1871, como sinônimo. Ora, justamente *T. briareus* se distingue desta última espécie de Verrill por não possuir depósitos calcáreos nas paredes do corpo. Por outro lado, havendo depósitos calcáreos nas paredes do corpo e sendo o anel calcáreo composto de dez peças simples mas com prolongamentos posteriores em *Thyone* (*Sclerodactyla*) *braziliensis*, estabeleceu-se diferença fundamental com *T. suspecta*. Além disso, a presença de dez tentáculos iguais em *T. suspecta* e oito iguais e dois menores em *T.* (*Sclerodactyla*)

braziliensis constituem também diferenças que, como as demais acima indicadas, justificam considerar esta última *Thyone* espécie válida.

Outras diferenças existem entre *T.* (*Sclerodactyla*) *braziliensis* e *T. briareus*, pois, nesta última, as placas do anel oral são três vêzes mais compridas e, além disso, o anel é composto de placas resistentes, com a parte posterior prolongada. Os pedicelos, espalhados por tôda a superfície, são muito menos numerosos do que em *briareus*.

Pelo tamanho, pelo aspecto dos tentáculos, número de vesículas de Poli, diferencia-se de *T. cognita*, e pelo número de tentáculos e caracteres do anel calcáreo distingue-se de *T. pervicax*. A ocorrência de dez tentáculos sendo dois ventrais menores aproxima-a de *T. belli* mas os caracteres da vesícula de Poli e do canal de areia constituem as diferenças fundamentais entre ambas.

Reivindicando, assim, a validez de *Thyone* (*Sclerodactyla*) *braziliensis* Verrill 1867, fazêmo-lo, todavia, com certas reservas pois, o gênero *Thyone* na opinião de abalizados autores (Ekmann 1925, pp. 100-101) é tido como artificial, visto conter elementos muito heterogêneos, opinião ainda mantida em 1938 por Deichmann (p. 376).

Finalmente, cumpre lembrar que *Thyone briareus* foi colocada na sinonímia de *Sclerodactyla briareus* (Leseur) por Panning (1949, p. 459) ao fazer a revisão da família Cucumariidae, classificação esta não aceita por alguns autores, entre os quais Cherbonnier (1952, p. 501).

OCORRÊNCIA

Recife dos Abrolhos; recifes da praia da Piedade, no litoral de Recife, Estado de Pernambuco.

5.

APODA

Chiridota rotifera Pourtalès 1851. Figs. 45-50

1867 *Chirodota rotifera* Pourtalès, Selenka — Zeits. wiss. Zool. v. 17, p. 367.

1867 *Chirodota rotiferum* Stimpson, Verrill — Trans. Conn. Ac. Art. Sci., vol. 1, pt. 2, p. 371, t. 4, figs. 9, 9a.

1879 *Chirodota rotifera* Stimpson, Rathbun — Trans. Conn. Ac. Art. Sci., v. 5, p. 141.

1882 *Chirodota rotifera* Stimpson, Ludwig — Mem. Cour. et Mem. Sav. étranger. Acad. Royale Belgique, v. 46, p. 25.

1910 *Chiridota rotifera* Pourtalès, Clark — Journ. Exp. Zool. v. 9, n. 3, p. 497, 2 tab. 6 figs., desenvolv.

1916 *Chirodota rotifera* Pourtalès, Sluiter — Zool. Jahrb., Abt. Syst. Suppl. 11, n. 2, pp. 331-341.

1930 *Chiridota rotifera* Pourtalès, Deichmann — Bull. Mus. Comp. Zool. Harvard, v. 71, n. 3, p. 212.

1939 *Chiridota rotifera* (Pourtalès), Engel — Capita Zoologica, v. 8, pt. 4, p. 11.

Em janeiro de 1956, no L.B.M. o Dr. Erasmo Garcia Mendes e o Lic. Chaim Grinkraut, ao capturarem animais durante um dos acentuados períodos de baixa-mar, tiveram oportunidade de recolher um exemplar de uma pequena holotúria rósea, cheia de filhotes, que mais tarde se identificou no gênero *Chiridota*. Posteriormente, em março e nos meses seguintes, capturaram-se numerosos outros exemplares.

DIAGNOSE

1. Corpo vermiforme, região anterior mais dilatada, região posterior afilada.
2. Tentáculos em número de 12, digitiformes nas extremidades, com 10 dedos, o par terminal mais longo.
3. Colorido varia de rosa ao vermelho. Depois de fixados em álcool, os exemplares ficaram amarelo-esbranquiçados.
4. Papilas distribuídas por todo o corpo.
5. Placas calcáreas em forma de roda de carroça, com 6 furos, agrupadas nas papilas.
6. Pele fina e áspera, devido às placas calcáreas.
7. Anel calcáreo formado por 10 peças simples sem prolongamentos.

DESCRIÇÃO

O material que examinamos constou de 18 exemplares (n.ºs 928 a 945) de 50 a 70 mm de comprimento e 2,5 a 5 mm de largura.

A pele é fina com papilas irregularmente distribuídas. Nas papilas encontramos os depósitos calcáreos em forma de roda de carroça (Fig. 46), com seis buracos. As papilas contêm de trinta e cinco a cinqüenta e cinco rodas.

Tentáculos em número de doze, digitiformes nas extremidades (Fig. 47), com dez dedos, sendo o par terminal mais longo.

Côr — Os animais vivos apresentavam um colorido que varia do rosa ao vermelho. Depois de fixado o material, apenas um conservou esta côr, os outros ficaram amarelo esbranquiçados.

A *Chiridota* é um gênero vivíparo.

Chiridota jovem. Figs. 49-50.

Um dos exemplares de *Chiridota rotifera* coletados em janeiro de 1956, na Ilhota do Baleeiro, possuia filhotes na cavidade do corpo, onde os ovos se desenvolvem. Apresentavam em geral 3 mm de comprimento e 1 mm de largura. São brancos, transparentes, com algumas papilas distribuídas pelo corpo, constituídas por placas calcáreas em forma de roda de carroça. Em cada rádio há seis papilas.

Na fase do desenvolvimento em que os animais foram descobertos, apresentavam êles oito tentáculos, não sendo possível contar o número de dedos por se encontrarem os tentáculos dobrados para o interior do animal.

Chama a atenção o estômago pelo seu desenvolvimento excepcional, e o canal digestivo que faz uma volta à altura do estômago, constituído do intestino que se prolonga depois em linha reta até o anus.

O anel calcáreo é formado por placas simples com as superfícies anteriores e posteriores quase paralelas, apresentando ligeiras saliências.

Forma — cilíndrica, com a extremidade posterior mais afilada que a anterior.

Côr — branca leitosa translúcida.

DISCUSSÇÃO

Como se vê na indicação da sinonímia o nome adotado para o gênero foi *Chirodota,* mas o nome correto é *Chiridota* tal como foi introduzido por Eschscholtz em 1829 segundo indicação de Deichmann (1930, p. 211).

Para determinação desta espécie seguimos esta autora e segundo a qual, *C. rotifera* difere de *C. larvae* por possuir bastonetes curtos e, de *C. pelorica* por serem, nesta, escassas as placas em forma de roda e os bastonetes curvos formarem aqui uma camada densa. Pelo aspecto, número e forma das placas, distingue-se de *contorta, australiana* e *japônica,* cujas diagnoses são bem explícitas e figuradas em Théel (1896, p. 15, t. 2, figs. 1-3). Por não ser dotada dos corpos em forma de semi-parêntesis difere de *gigas, rígida* e *magna,* conforme descrição de Clark (1921, p. 162; 1938, p. 555) e de *geminifera* (Dendy e Hindle 1907, p. 112), por esta ser desprovida de placas em forma de rodas e possuir os bastonetes sigmoides ausentes naquela. Além disso, a forma e o aspecto das rodas de carroça são bem característicos. Como se sabe, estas rodas são elemento de importância na classificação das *Synaptidae* (Ludwig 1892, p. 350).

OCORRÊNCIA

Costa leste da América do Norte, Baía de Biscaia (Flórida), Barbados, Tortuga, Jamaica, Curaçao, Bonaire, Abrolhos, São Sebastião (Praia do Segrêdo, Ilhota do Baleeiro em frente ao L. B. M.). Encontram-se sob as pedras que se descobrem na baixa-mar ou enterradas na areia.

6.

Synaptula secreta sp. nov. Figs. 51-56

Em setembro de 1956, ainda no L.B.M., durante os exercícios do curso de Biologia Marinha, ao capturar animais, os estudantes adiantados, principalmente a aluna Hortencia Maria Gomes, aos quais agradecemos o valioso auxílio, recolheram 14 exemplares de uma pequena holotúria que posteriormente se verificou pertencer ao gênero *Synaptula*. Mais tarde, em outras ocasiões, outros

exemplares (4) foram por nós recolhidos, notando-se ser material muito freqüente naquêle local. (N.ºs 910 a 927).

DIAGNOSE

1. Corpo vermiforme, extremidade anterior mais ampla, parte posterior mais afilada.
2. Dez tentáculos, digitiformes, apresentando cinco dedos.
3. Bôca invaginada.
4. Pele fina áspera, devido às placas calcáreas.
5. Placas calcáreas em forma de âncora e placa em forma de escudo.
6. Papilas distribuídas por todo o corpo.
7. Anel calcáreo formado por 10 peças simples. Junto a êste um outro anel de suporte, composto de denso tecido conjuntivo, chamado anel cartilaginoso.

DESCRIÇÃO

Corpo vermiforme, extremidade anterior mais dilatada, afilando-se posteriormente (Fig. 51). Dimensões: de 5 a 25 mm.

Côr — Alguns animais fixados apresentam-se rosados e outros esbranquiçados. Os animais que se apresentam rosados, possuem as papilas de uma tonalidade bastante escura, côr de ferrugem. Os animais vivos são claros com tonalidade avermelhada devido à presença de numerosas papilas também côr de ferrugem.

Tentáculos digitiformes (Figs. 51 e 53), em número de dez, com cinco dedos, de um mm de comprimento cada um.

Pele fina e transparente, porém áspera.

Bôca invaginada com 0,2 mm de diâmetro num animal de 10 mm de comprimento.

Papilas arredondadas, distribuídas por todo o corpo do animal alternando com as placas calcáreas e sobressaindo bastante na pele. Anel calcáreo de peças juxtapostas, formando um conjunto denteado (Fig. 56).

Vesículas de Poli em número de três, sendo uma maior e duas menores de igual tamanho (Fig. 52).

Âncoras com haste de contornos lisos e não ramificada (Fig. 55); os braços são lisos, o que ocorre também com o vertex. Placas das âncoras (Fig. 54) com dois grandes buracos centrais, circundados por cinco outros menores. Todos êsses buracos têm as bordas completamente lisas. Na extremidade posterior estreita da placa, há dois buracos menores do que os outros, pelos quais um arco bem distinto da âncora cruza a superfície da placa.

Ao longo de certos interradios, de 2 no caso, encontramos internamente urnas distribuídas por tôda a extensão, presas à parede do corpo, da parte anterior à parte posterior, por um pedúnculo de tecido conjuntivo.

As âncoras (Fig. 55) são tôdas iguais e do mesmo tamanho, desde a região anterior à extremidade posterior contràriamente ao que assinala Cuénot (1948, p. 119) neste particular. Os braços são lisos, não ramificados e desprovidos de botões; placas das âncoras (Fig. 54) com dois grandes buracos centrais circundados por cinco outros laterais menores, todos de bordas lisas; na extremidade posterior mais estreita da placa há dois buracos lisos pelos quais passa uma alça, o interior da qual é atravessado pela haste da âncora.

Os músculos longitudinais são formados por apenas uma faixa e não duplos como se observa em outras holotúrias.

A ocorrência de papilas ferrugíneas, devida à desusada concentração de pigmentos na pele desta holotúria, parece estar em relação com o teor de ferro. A presença dêste metal relaciona-se com a quantidade do mesmo existente no local onde os animais habitam, tal como acontece com *Synapta roseola* conforme indicação de Clark (1901, p. 25).

DISCUSSÃO

O gênero *Synaptula* foi descrito por Oersted em 1849. Clark (1907, p. 80, ap. Deichmann 1930, p. 205) indica como características principais as seguintes: "dez a quinze tentáculos pinulados; pelo menos cinco dedos de cada lado; anel calcáreo presente; três ou mais vesículas de Poli; canal de areia simples, não ramificado. Órgãos dos sentidos em forma de olhos pigmentados na base dos tentáculos no disco oral quando presente. Haste da âncora fina-

mente denteada mas não ramificada. Braços lisos, mas o vertex com alguns nós diminutos; placas das âncoras com um grande buraco central, circundado por seis outros buracos, todos mais ou menos denteados, e com dois grandes buracos lisos na extremidade posterior estreita, onde um arco bem formado e distinto cruza a superfície da placa".

Êsses caracteres encontram-se quase todos no material que obtivemos da Praia do Segredo e daí o incluirmos no gênero *Synaptula.*

O fato de apresentarem as nossas *Synaptula* os contornos das âncoras lisas, e assim também os dos buracos das placas das âncoras, levou-nos a considerar esta *Synaptula* uma espécie nova.

Por não possuirem dentículos as âncoras da espécie nova, e não serem denteadas as bordas dos buracos das placas das âncoras, distingue-se esta espécie das demais espécies conhecidas de *Synaptula,* i. é., *vivipara, hydriformis, nigra, psara, recta, reticulata, picta.* Por outro lado, o tamanho diferencia-a de *S. reticulata* que mede 20 cm. O número de tentáculos (10) serve para separá-la de *S. psara* e *S. recta*. A presença de uma faixa negra dos tentáculos em *S. nigra,* ausente em *S. secreta* constitui também distinção entre ambas (Clark 1921, p. 160; 1924, pp. 473-477).

Synaptula secreta difere de *S. rubra* pelo tamanho pois esta mede 18 cm (Heding 1931, p. 655) e pelo número de tentáculos. Em *rubra* há 12-13 tentáculos com 16 pares de dedos e, além disso, o anel calcáreo é de aspecto bem diferente. Por outro lado, *S. rubra* possui 20 vesículas de Poli e as âncoras são ligeiramente denteadas. Comparadas as figuras de Heding (l. c., Figs. 1-11) com as do nosso material, não há dúvida quanto às diferenças. De *S. denticulata* diferencia-se também pelo tamanho (13 cm) e número de tentáculos (13), sendo que aqui os dedos dos tentáculos se acham ligados por membranas. De *S. boweniensis,* pelo tamanho (10 cm) e número de tentáculos (13 cm 5-20 pares de dedos), pelo denteadura das âncoras e irregularidade das bordas do anel calcáreo. De *S. tualensis,* pelo comprimento (2 cm) aproxima-se de *S. secreta,* mas dela difere pelo número de tentáculos e pela presença de membrana interdigital. Além disso o número de vesículas de Poli (12) é maior. De *S. purpura,* pelo comprimento (5 cm) e pela forma de anel calcáreo, e ainda pelo número de vesículas de Poli (20 gran-

des e 5-8 pequenas), embora se aproxime pelo número de tentáculos (10).

Convém lembrar que o gênero *Synaptula* de Oersted 1849, foi incluído por Clark (1896, p. 400) na sinonímia de *Synapta* ao descrever uma *Synapta vivipara* das Índias Ocidentais, relembrando que Oersted em 1850 a indicou como *Synaptula vivipara*. Logo a seguir Clark (l. c.) transcreve a opinião de Ludwig (1881, ap. Clark l. c.) que diz: "deve haver algum melhor fundamento para distinção genérica, que o estado no qual os jovens nascem, e, faltando êste, *Synaptula* não permanece como um gênero e torna-se sinônimo de *Synapta*".

O próprio Clark, porém, faz ressurgir o gênero (1910, p. 496) indicando *Synapta vivipara* (Oerst.) na sinonímia de *Synaptula hydriformis*.

Acontece, ainda mais, que Deichmann (1930) ao estudar as holotúrias da parte oeste do Atlântico faz a revisão dos *Apoda* e, ao tratar das *Synaptidae* (p. 204) reinclui o gênero *Synaptula* ao lado de *Euapta*, de *Leptosynapta* e *Protankyra*, tirando-o da sinonímia acima referida. Acompanhamos Deichmann (l. c.) neste particular, e também para a determinação do nosso material.

Por outro lado, comparando as características do material que temos em mão com o dos vários autores que mostram os desenhos das partes tomadas para classificação (principalmente âncoras, placas das âncoras, tentáculos) não temos dúvida que se trata de material diferente.

Seja-nos ainda permitido lembrar aqui a existência de uma certa confusão na taxonomia das *Synaptidae*. Assim, Selenka (1867, p. 365) enumera *Synaptula vivipara* Oerst. logo em seguida às descrições das várias espécies de *Synapta* (p. 360 e seg.) sem dar-lhe, porém, a descrição e Fisher (1907), ao tratar das holotúrias do Hawaii, mantém o gênero *Synaptula* Oerst. (p. 717) indicando como tipo *S. vivipara* Oerst. justamente, o contrário do que fêz Clark (1886, p. 400) apoiado em Ludwig.

Sem dúvida, o fenômeno da viviparidade, característico de *Synaptula vivipara*, deve ter chamado tanto a atenção dos autores que os mesmos deixaram de lado outros caracteres não menos impor-

tantes para diagnose do gênero, como sejam tipo das âncoras, das placas de âncoras, morfologia dos tentáculos, etc.

Realmente, a questão é complexa, pois tais caracteres também ocorrem por vêzes em *Leptosynapta* e em *Heterosynapta* conforme a descrição de Verrill (1867, pp. 325 e 346), e em parte em *Chondrocloea*, como se lê em Sluiter (1904, p. 125).

Depois que se verificou o interessante fenômeno da viviparidade e o cuidado dos filhotes também em outras holotúrias, *Cucumaria glacialis* (Mortensen 1897, p. 717), *Chiridota contorta* (Ludwig 1897, p. 237), *Cucumaria planci* (Gerould 1898, p. 273), *Thyone rubra* (Clark 1901a, p. 166), *Chiridota rotifera* (Clark 1910, p. 495), *Leptosynapta minuta* (Mortensen 1927, p. 427; 1928, p. 107), *Synaptula vittata* (Mortensen 1938, p. 49), deixou o mesmo de ser elemento único para diagnose do gênero.

Dos outros característicos levados em conta em tal diagnose prevalecem o número dos tentáculos, sua forma, presença de botões digitiformes, forma e disposição das âncoras e das placas calcáreas.

Já em 1893, tratando de *Synapta reciproquans* Forskal, Hérouard acenara para as dificuldades da determinação de caracteres distintivos destas espécies, dizendo (p. 137): "Il est à présumer qu'un jour toutes ces espèces, ainsi que *Synapta indivisa* Semper seront rapportées à la *S. reciproquans* et ne subsisteront plus que comme varieté. Leurs caractères sont en effect à peu identiques, si ce n'est la variabilité dans le nombre des tentacules; mais rien ne dit qu'on ne reconnaïtre pas un jour que, chez les *Synaptes*, le nombre des tentacles croït pendant le développement post-embryonnaire. Le nombre variable (14-16) des tentacules de *Synapta Godeffroyi* Semper est un acheminement vers la conviction de ce fait.

Les ancres présentent des anomalies assez nombreuses, résultant dans la bifurcation de leur tige (fig. 2); les plaques à ancres présentent une déformation correspondante".

Quer-nos parecer que o estudo posterior de numerosas espécies veio demonstrar que, se na realidade o número de tentáculos varia, a sua forma e aspecto são relativamente constantes. Além disso, deve-se levar em conta que nas numerosas diagnoses de várias espécies de *Synaptula*, a forma das âncoras e a das placas das âncoras aparecem relativamente constantes para cada espécie como se

pode verificar pelos estudos ulteriores que mostraram não ser tão sensível a variação da forma das âncoras e das placas das âncoras, como se depara nas descrições de Clark (1901, p. 27) para as *Synapta* e na de Fisher (1907, p. 720).

Os caracteres aqui considerados autorizam-nos a considerar a *Synaptula* descrita, como espécie nova. A designação *secreta* refere-se ao local de captura, i. é, Praia do Segredo, sede do L.B.M.

7.

NOTAS BIOLÓGICAS

Durante a colheita das holotúrias quase sempre na zona das marés, na baixa mar, conseguimos fazer algumas observações que, em geral, se completavam nos laboratórios.

Em geral, as praias que percorremos para a referida colheita eram praias duras, com abundantes rochas.

E' hábito considerar as holotúrias como bentônicas. Poucas são as capazes de nadar livremente, como *Stichopus natans*, que pela primeira vez foi observada por Sars em 1867 (ap. Ludwig 1892, p. 415; Hansen e Hansen 1956, p. 55) nadando livremente, e, assim, elevando-se do fundo do mar. Segundo o referido autor, os movimentos assemelham-se aos das sanguessugas e aos de certas planárias. Observações semelhantes foram feitas por Gilchrist (1920, p. 381) com holotúrias que vivem na profundidade nas costas sul-africanas.

Há as espécies pelágicas e as que vivem no fundo do mar, aderentes a diversos substratos ou enterrando-se na areia. São encontradas desde a superfície do mar até a profundidade de ca. de 7.000 metros, zona em que recentìssimamente foram capturadas durante a nova Expedição da Galathea (Kramp 1953, p. 79; Bruun 1953, p. 183; Hansen 1956, p. 33).

Como se sabe, *Holothuria grisea* vive em biótopo bastante característico, fixando-se firmemente nas pedras, nas reentrâncias ou depositadas sôbre a areia e conchas. Retiradas dêsse ambiente, logo aumentam consideràvelmente o turgor, o qual se pode perfeitamente verificar comprimindo-se o animal entre os dedos. Deixadas nestas condições no aquário, pouco a pouco relaxam os mús-

culos e, quando em completo repouso em aquário bem arejado, distendem-se, chegando a alcançar até 40 cm de comprimento. Não irritadas, emitem os tentáculos arborescentes, os quais se recolhem sob qualquer excitação direta do animal.

A ejeção das vísceras dá-se quando as condições do aquário se tornam bem precárias, principalmente no que se refere à falta de oxigênio.

Outras observações pudemos fazer com *Stichopus badionotus*. São animais de relativa profundidade. Todos os que obtivemos foram capturados durante o mergulho até 4 m. O corpo eriçado de saliências negras sob fundo róseo-alaranjado, dá ao animal um aspecto especial, donde o nome popular de "diabo do mar". No aquário, em repouso, emite os tentáculos e distende enormemente o corpo chegando a medir até 60 cm de comprimento por 10 de largura. Tomado com as mãos retrai-se imediatamente, reduzindo-se à ca. de 30 cm de comprimento e secretando abundante mucosidade, que a torna escorregadia. Segundo Cuénot (1940, p. 85), o tecido conjuntivo de diversas espécies de holotúrias (notadamente *Stichopus*), algumas horas após a morte, reduz-se a uma mistura de mucina e condrina.

No que se refere à ejeção das vísceras, parece-nos que êsse fenômeno, é mais freqüente em *Stichopus badionotus* que em *H. grisea* pois todos os exemplares capturados pouco tempo depois expulsavam os intestinos. O mecanismo de evisceração foi estudado em *Holohutria tubulosa* e em *Stichopus regalis* por Bertolini (1930, p. 439; 1933, p. 434) e, segundo esta autora, o fenômeno de regeneração das vísceras (1933, p. 432) não é um fenômeno acidental, mas parece ligado a condições biológicas dêstes animais que acabam por expelir periòdicamente o intestino, a rêde admirável dos "pulmões", para regenerá-los logo em seguida. Assim, ainda de conformidade com a referida autora (l. c.) pelo menos em *Stichopus regalis*, a evisceração é um processo natural, ligado às condições naturais de vida e difere do de outras espécies como as do gênero *Holothuria*, nas quais a emissão dos intesitnos e a sucessiva regeneração não são fenômenos normais.

E' interessante notar que durante o período que se segue à evisceração, os *Stichopus* permanecem completamente privados dos

chamados pulmões, e nesse período os seus órgãos respiratórios devem ser outros e verossìmilmente representados pela pele, pelos pedicelos ambulacrais e pelos tentáculos bucais (Bertolini 1933a, p. 9).

Enquanto que *H. grisea* pode permanecer até meses no aquário, mesmo em condições não muito favoráveis, *Stichopus badionotus* não é resistente, não chegando a sobreviver nem doze horas no aquário, ainda que as condições sejam tidas por ótimas. Além disso, se fôr retido nesse ambiente, o animal passa logo a decompor-se de modo que para uma boa fixação se deve ter a preocupação de o anestesiar imediatamente após a captura. Talvez devido à pequena resistência dêstes animais, os músculos longitudinais perdem muito cedo o seu poder de contração, sendo pois material de difícil manuseio e de parca utilização em fisiologia.

Ainda quanto à resistência para se manter no aquário, é sabido que as *Dendrochirota* são mais resistentes que as *Aspidochirota* e *Apoda* (Ludwig 1892, p. 422).

Achado bem interessante vem a ser o da *Chiridota rotifera* com numerosos embriões. Tendo havido ruptura da pele do animal, grande número de embriões saiu, restando apenas alguns poucos na cavidade do corpo do animal. Infelizmente, o material veio às nossas mãos já fixado, não possibilitando observações sôbre os embriões vivos. Registramos o mês de janeiro em que se capturou o referido exemplar, como época de reprodução da espécie. O estudo de Clark (1910, p. 479) referente a esta holotúria é bastante completo, embora deixe ainda alguns pontos em claro. Numa resenha sôbre as holotúrias vivíparas, Gerould (1898, p. 278) afirmou ter notado em *S. vivipara* degeneração do duto genital, afinamento das paredes dos túbulos reprodutores e abertura através da parede do reto. Infelizmente, o nosso material não possibilitou verificar estas alterações, mas podemos afirmar que o duto genital ainda se achava presente na *Chiridota* que estudamos.

Finalmente, examinando o material, verificamos que muitas *Chiridota* apresentavam o corpo recoberto por numerosos pequenos animais, achatados, de ca. de 0.5 mm de comprimento, fixos pela extremidade anterior.

Como se sabe (Ludwig 1892, p. 429; Hyman 1955, p. 240) numerosos são os metazoários que vivem em associação com holotúrias, principalmente Tubelários rhabdocelos da família *Umagillidae*. Desta família, segundo Hymann (l. c.) *Anoplodium chirodotae* vive no celoma de *Chiridota laevis*. No nosso material, porém, trata-se de ectomensais ou ectoparasitos muito pequenos, cujo gênero ainda não conseguimos identificar. Achavam-se êles espalhados por tôda a superfície do corpo da holotúria, aglomerando-se principalmente na base dos tentáculos. Trata-se certamente de rotatórios, a serem estudados oportunamente.

As observações sôbre *Thyone* (*Sclerodactyla*) *braziliensis* foram-nos relatadas pessoalmente pelo Prof. Paulo Sawaya, que as capturou nos arredores de Recife, Estado de Pernambuco. O material foi colhido na baixa mar quando os recifes se achavam descobertos em grande extensão. Com um martelo quebraram-se algumas partes de modo a se descobrirem as locas em que jazem, comunicantes por pequenas aberturas com o mar. Havia nas locas boa quantidade de ouriços do mar, ofiúros, algumas estrêlas, numerosos poliquetos e holotúrias, sendo abundantes as *H. grisea* e escassas as *Thyone*. Retiradas estas das locas, pouco se contrairam. Deixadas num recipiente grande com água do mar arejada distenderam-se mais um pouco e no dia seguinte expulsaram os *Carapus*.

A forma destas holotúrias no estado de repouso não difere muito do contraido, sendo em ambos oval.

As *Synaptula secreta* vivem, como outras holotúrias, ora aderentes a diversos substratos, ora jazendo na areia e recobertas pelas rochas. Quando se retiram os animais dêsse ambiente e são depositados numa placa de vidro com água do mar, começam a apresentar constrições das paredes do corpo, a partir da extremidade anterior. Em certos casos, tais constrições são tão fortes que o animal chega a autotomizar-se, dividindo-se o corpo em numerosos fragmentos. Tais constrições que terminam na autotomia, são precedidas de acentuado alongamento do corpo, chegando a triplicar o seu comprimento. O animal passa a apresentar, nesse estado, o aspecto de uma longa fita estreita e bastante delgada. Como se sabe, constitui característico dos *Apoda* a perda da parte posterior do corpo quando ir-

ritados, bastando, às vêzes, apenas tomá-los com as mãos (Deichmann 1947, p. 326) para que isso ocorra.

Como acontece nas *Synapta,* em geral, a secção dos fragmentos inicia-se pela extremidade posterior e o côto anterior pode ficar vivo e regenerar o animal (Cuénot 1949, p. 88).

Estas observações feitas com *S. secreta* coincidem em grande parte com as de Clark (1899, p. 25) em *Synapta inhoerens* da Nova Inglaterra. Diz o autor que são condições patológicas e o comportamento dos animais, principalmente no que se refere à autotomia, deve-se em primeiro lugar à falta de oxigênio e de substrato adequado. Não se trata de fenômeno normal ou defensivo, pois se os animais forem colocados em recipiente com areia e houver arejamento conveniente, nela se enterrarão e aí poderão viver durante muito tempo. As obervações feitas até agora correspondem apenas ao comportamento de *S. secreta* trazidas do L.B.M. em condições, agora sabemos, relativamente precárias de manutenção, e daí o fato de pouco terem resistido no laboratório do Departamento em São Paulo.

A julgar pelo que o ocorreu com *Chiridota rotifera* obtida do mesmo local, trazida em frasco com areia no fundo podendo permanecer vivas longo tempo no laboratório, queremos crer com *S. secreta,* que vive no mesmo biótopo, se dê o mesmo.

No que se refere à viviparidade ainda não podemos saber se êsse fenômeno ocorre no nosso material. Observações futuras e manutenção em aquários em condições adequadas poderão talvez elucidar êste ponto.

8.

RESUMO

Estudaram-se várias holotúrias do litoral brasileiro, na extensão entre Cananéia, Estado de São Paulo, e Recife, Estado de Pernambuco. O material mais abundante foi colhido em Santos e em São Sebastião, no Laboratório de Biologia Marinha, localizado na Praia do Segrêdo, também chamada Cabelo Gordo de Fora.

Determinaram-se as seguintes espécies:

1. Ordem — *Aspidochirota*:
   Holothuria grisea Selenka 1867
   Sitchopus badionotus Selenka 1867

2. Ordem — *Dendrochirota*:
Thyone (*Sclerodactyla*) *braziliensis* Verrill 1867
3. Ordem — *Apoda*:
Chiridota rotifera Pourtalès 1851
Synaptula secreta sp. nov.

Assinalou-se o limite sul de distribuição de *Holothuria grisea* até agora verificado, que compreende a região de Cananéia, no litoral do Estado de São Paulo.

Stichopus badionotus é apontada pela primeira vez no litoral brasileiro, e *Chiridota rotifera* pela segunda.

O reencontro em Recife de *Thyone* (*Sclerodactyla*) *braziliensis* colhida nos arredores dos Abrolhos pelo Dr. C. F. Hartt e descrita em 1867 por Verrill, é fato digno de nota que indica a distribuição desta holotúria nas regiões equatorial e temperada em biótopos característicos. O gênero tem larga distribuição, desde Terra Nova, sendo muito abundante nas Caraibas. Na costa brasileira foram assinaladas *Thyone* em Fernando de Noronha (*Thyone cognita*), Bahia (*T. pervicax*) e Abrolhos (*T. Belli*).

Fato digno de nota vem a ser a ocorrência do conhecido peixe *Carapus* (= *Fierasfer*) comensal desta holotúria, conforme publicação feita em 1956 por Ancona Lopez.

A obtenção de *Chiridota rotifera* na Praia do Segrêdo, litoral de São Sebastião, mostra a extensão da distribuição geográfica desta espécie, também interessante por ser vivípara. A oportunidade de ter em mãos um exemplar portador de cêrca de 80 filhotes possibilitou registrar a ocorrência e descrever numèricamente a morfologia dos embriões, confirmando assim as interessantes observações de Clark (1910, p. 497).

O L.B.M. foi tomado como base de estudos, procurando-se fazer o inventário da fauna local (Praia do Segrêdo). A região é rica principalmente em Equinodermes. No material colhido sistemàticamente identificou-se uma nova espécie, *Synaptula secreta*. Descreveu-se com pormenores a morfologia e fêz-se ampla discussão da espécies. A presença de âncoras e placas de âncoras de borda inteiramente lisas com vários outros caracteres, justificam a nova espécie.

A revisão da bibliografia levou à consideração de vários pontos interessantes, dentre os quais se sobressai a ecologia dêstes Equinodermes, principalmente em suas relações com a bioquímica comparada. Assim, por exemplo, a coloração ferrugínea dos animais parece relacionar-se com o teor em ferro do ambiente, fato que ainda aguarda confirmação, pelo menos para os animais da Praia do Segrêdo.

Além da descrição das referidas espécies adicionaram-se numerosas notas biológicas relacionadas pràticamente com a ecologia de cada uma delas.

9.

SUMMARY

Some species of Holothurians from the Brazilian coast have been described, between Cananéia on the south and Recife, on the north, Capital of the State of Pernambuco.

The most part of holothurians was captured at the Marine Biological Laboratory at the "Praia do Segrêdo" (Segrêdo beach). Another local of study of those Echinoderms was the bay of Santos, State of São Paulo.

The following species were determined and described: *Holothuria* (*Holothuria*) *grisea* Selenka 1867, *Stichopus badionotus* Selenka 1867; *Thyone* (*Sclerodactyla*) *braziliensis* Verrill 1867; *Chiridota rotifera* Pourtalès 1851; *Synaptula secreta* sp. nov.

The southernest limit checked of the distribution of *Holothuria* (*Holothuria*) *grisea* was the region of Cananéia, on the littoral of São Paulo.

Stichopus badionotus has been now captured by the first time on the Brazilian coast, and *Chiridota rotifera* by the second time.

Thyone (*Sclerodactyla*) *braziliensis* was found in the reefs of the littoral of Recife, on the north. This holothurian was obtained by the first time from the reefs of Abrolhos by Dr. C. F. Hartt and described by Verrill in 1867. It occurs in the tropical and temperate regions on characteristic biotops. The genus *Thyone* is largely distributed, from Newfoundland, and is very common at the Caribbean region. On the Brazilian coast only three *Thyone* were

captured up to now: in Fernando de Noronha island (*Thyone cognita*), in Bahia (*Thyone pervicax*) and in Abrolhos (*Thyone belli*).

It has been noted that the very interesting *Carapus* (= *Fierasfer*) a commensal fish, is carried out by the *Thyone* (*Sclerodactyla*) *braziliensis*. That fish was previously described by Ancona Lopez (1956).

Another holothurian found by the second time at the Brazilian coast is the viviparous *Chiridota rotifera,* captured at the Praia do Segrêdo, near São Sebastião. One specimen collected under the rocks at the low-tide was full of embryos (80). The description of the embryos is given in this paper. This observation confirms those of Clark (1910, p. 497).

Synaptula secreta here studied is a new speceis caught under the small rocks exposed at the low tide at the "Praia do Segrêdo". On September of 1956 eighteen specimens were obtained.

Diagnosis — Body vermiform, anterior extremity broader than the posterior. Dimensions: 5 to 25 mm long.

Colour — Some fixed specimes are reddish and some others whitish. The reddish animals have the body covered by several dark red papillae (Figs. 51 - 56). Each has ten digitiform tentacles with five fingers of 1 mm of length. Skin thin and transparent, but rough. Mouth invaginated with 0,2mm. of diameter in one animal of 100mm in length. Round papillae cover all the body surface appearing as small eminences and alternate with the calcareous plates. Calcareous ring made by connected pieces, with dentate aspect. Simple anchors with smooth flukes distally tapered describing an arc equal of 0,6mm. of the length of the central shaft which terminates distally in a short bar with smooth edge. Anchor stick also with smooth edges and of 0,5mm of the length Anchor plates oval, about 0.4 to 0.45 mm with a narrowed prolongation supporting the handle; the disk pierced by seven large, somewhat unequal, subcircular holes, all with smooth edges. Two holes are larger than the other and placed in the center of the disk. The other five holes are distributed around those central. On the narrowed part of the handle there are two holes smaller than the others. They are symmetrically placed and separated by the narrow apex provided with two teeth. The anchors and the

anchors plates are very numerous (about forty sq.mm) in the skin; these pairs being separated from one another by an average space of one and a walf to twice the length of the anchors. The anchors (Fig. 55) are of the same size and distributed uniformely from the anterior to the posterior extremity. Arms of the anchors are smooth and simple, not branched, and lacking of buttons. There are no miliary rosettes. Single longitudinal muscles. The calcareous ring has ten pieces, each without perfuration or notch.

Geographical distribution and ecological aspects of all holothurians here described were also considered.

10.

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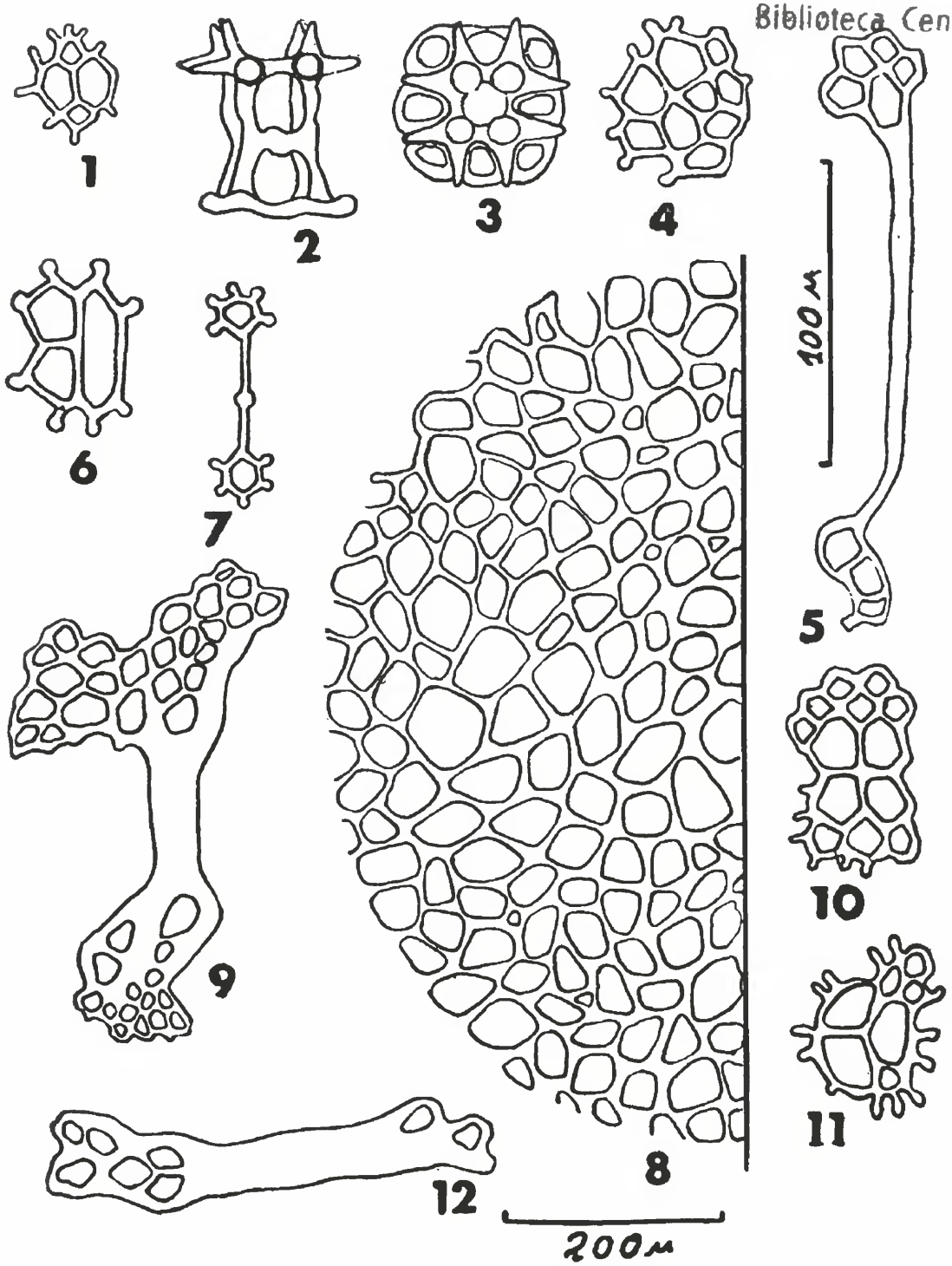
ESTAMPA I

INDICAÇÃO DAS FIGURAS

Holothuria grisea Selenka

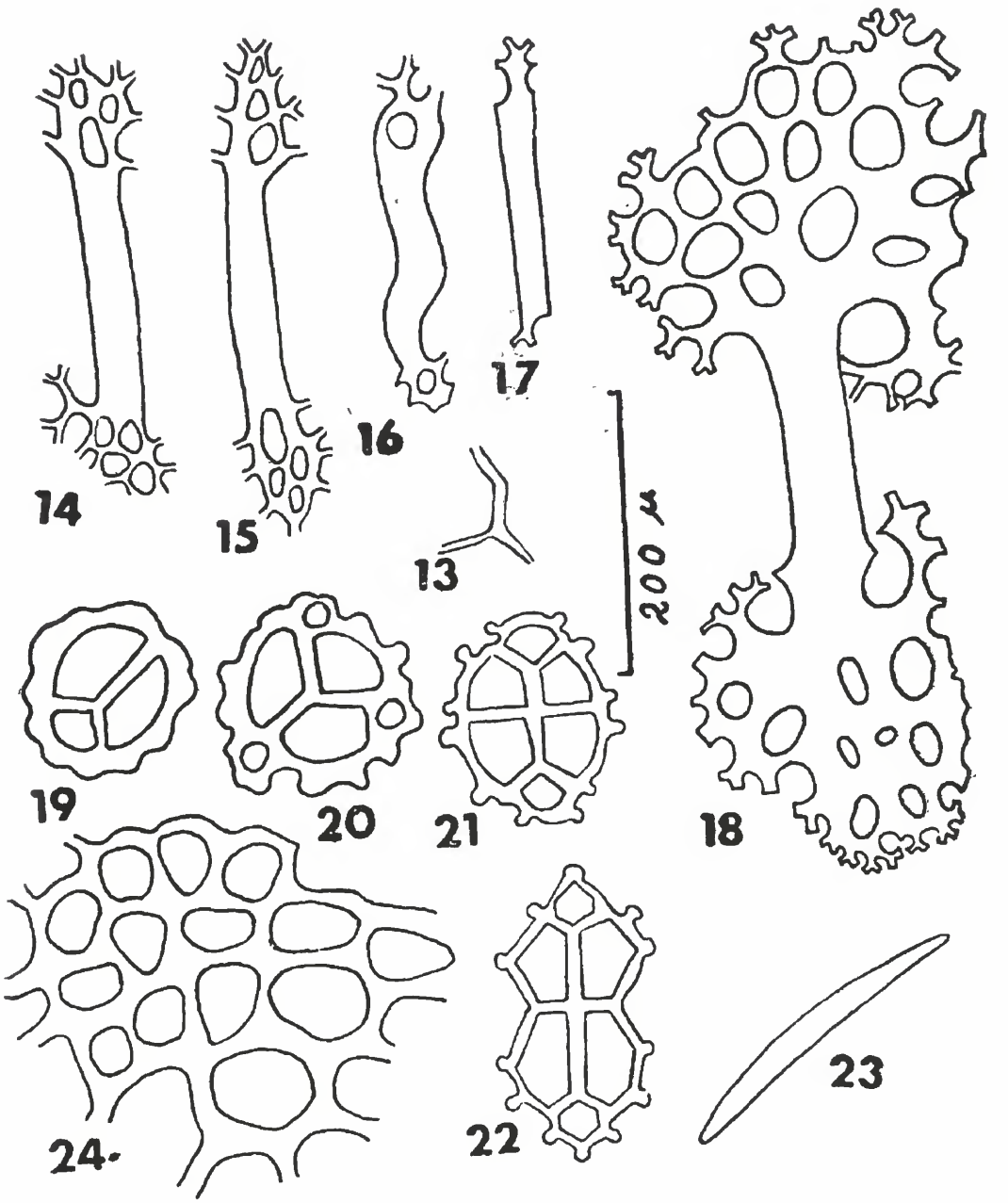
N.º 1 — Placa fenestrada.
N.º 2 — Processo em forma de tôrre, vista lateral.
N.º 3 — Processo em forma de tôrre, visto de cima.
N.º 4 — Roseta ramificada.
N.º 5 — Bastonete com extremidade ramificada.
N.º 6 — Placa fenestrada.
N.º 7 — Bastonete com extremidade ramificada.
N.º 8 — Disco.
N.º 9 — Bastonete com extremidade ramificada.
N.º 10 — Placa fenestrada.
N.º 11 — Roseta ramificada.
N.º 12 — Bastonete com extremidade ramificada.



ESTAMPA II

Holothuria grisea Selenka

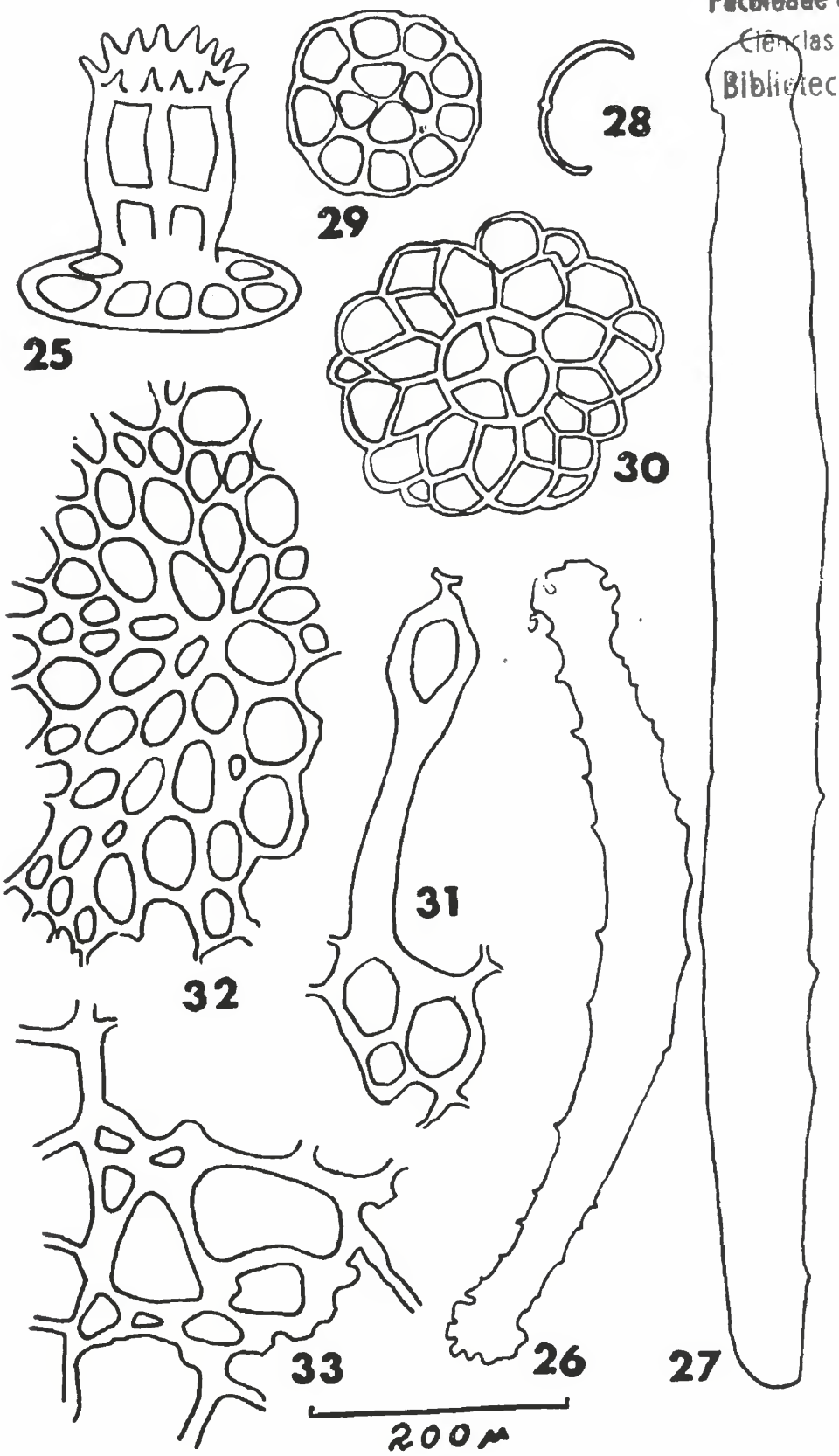
N.º 13 a 18 — Bastonetes com extremidades ramificadas.
N.º 19 e 20 — Processos achatados semelhantes a rosetas.
N.º 21 e 22 — Placas fenestradas.
N.º 23 — Bastão de apôio.
N.º 24 — Fragmento de disco.



ESTAMPA III

Stichopus badionotus Selenka

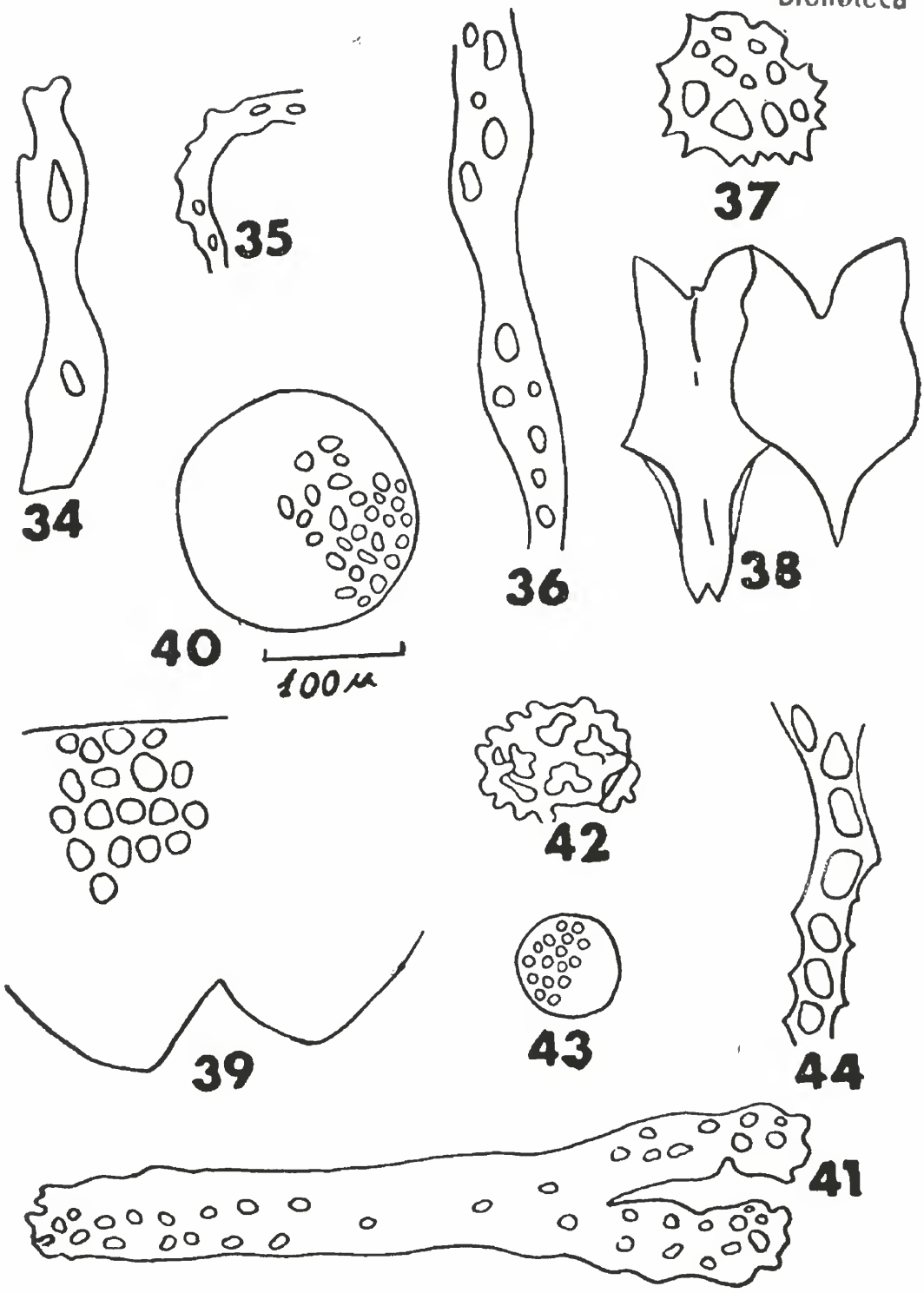
N.º 25 — Tôrre.
N.º 26 — Bastão delgado.
N.º 27 — Bastão espinhoso.
N.º 28 — Placa em forma de C.
N.º 29 — Tôrre vista por baixo.
N.º 30, 32 e 33 — Processos reticulares.
N.º 31 — Bastão reticular.



ESTAMPA IV

Thyone (*Sclercdactyla*) *braziliensis* Verrill 1867

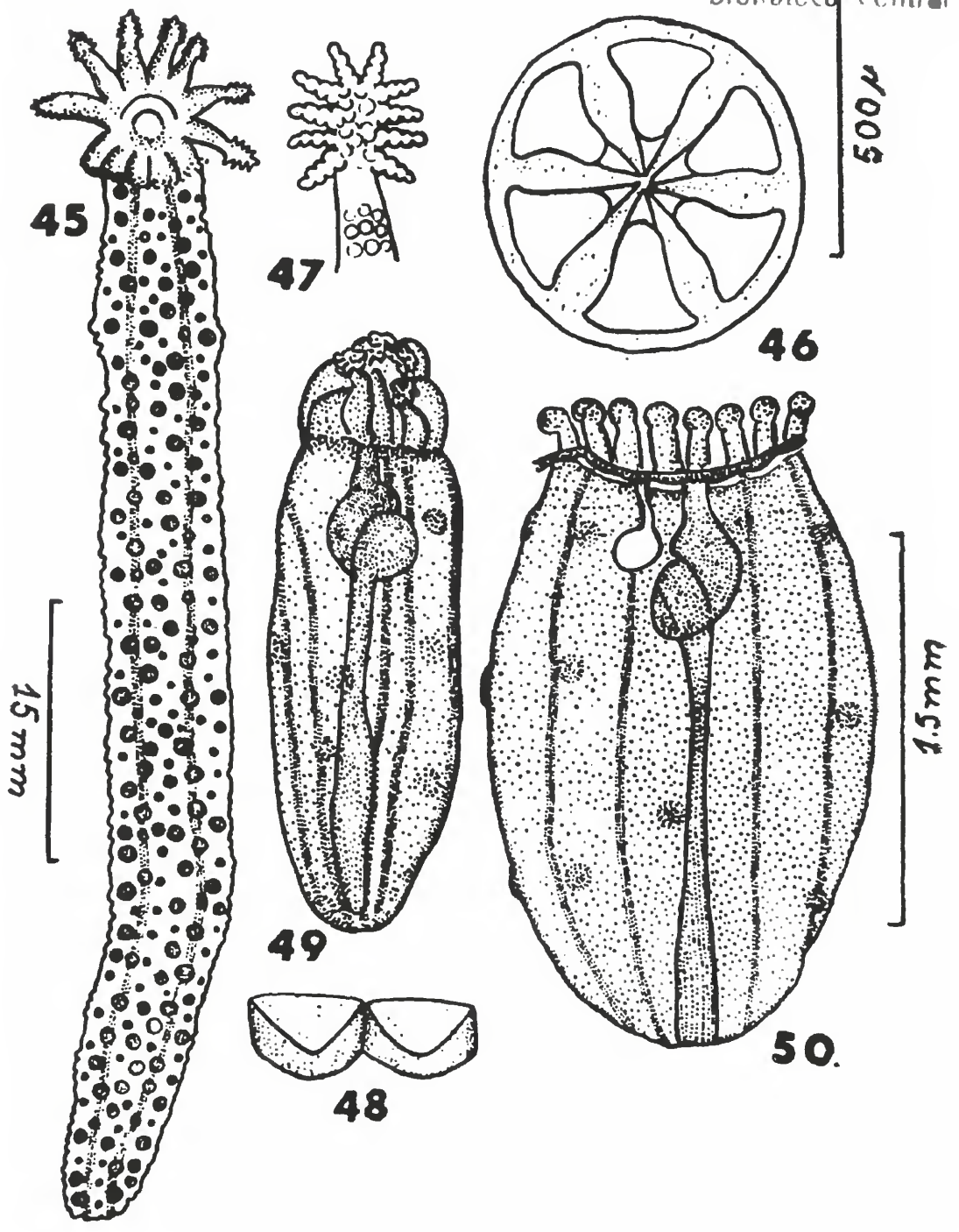
N.º 34-35 — Placa curva de suporte.
N.º 36 — Bastão delicado.
N.º 37 — Roseta.
N.º 38 — Dentes do anel calcáreo.
N.º 39 — Dentes do anus.
N.º 40 — Placa ou disco terminal.
N.º 41 — Bastonete.
N.º 42 — Taça.
N.º 43 — Disco terminal.
N.º 44 — Bastonete.



ESTAMPA V

Chiridota rotifera Pourtalès 1851

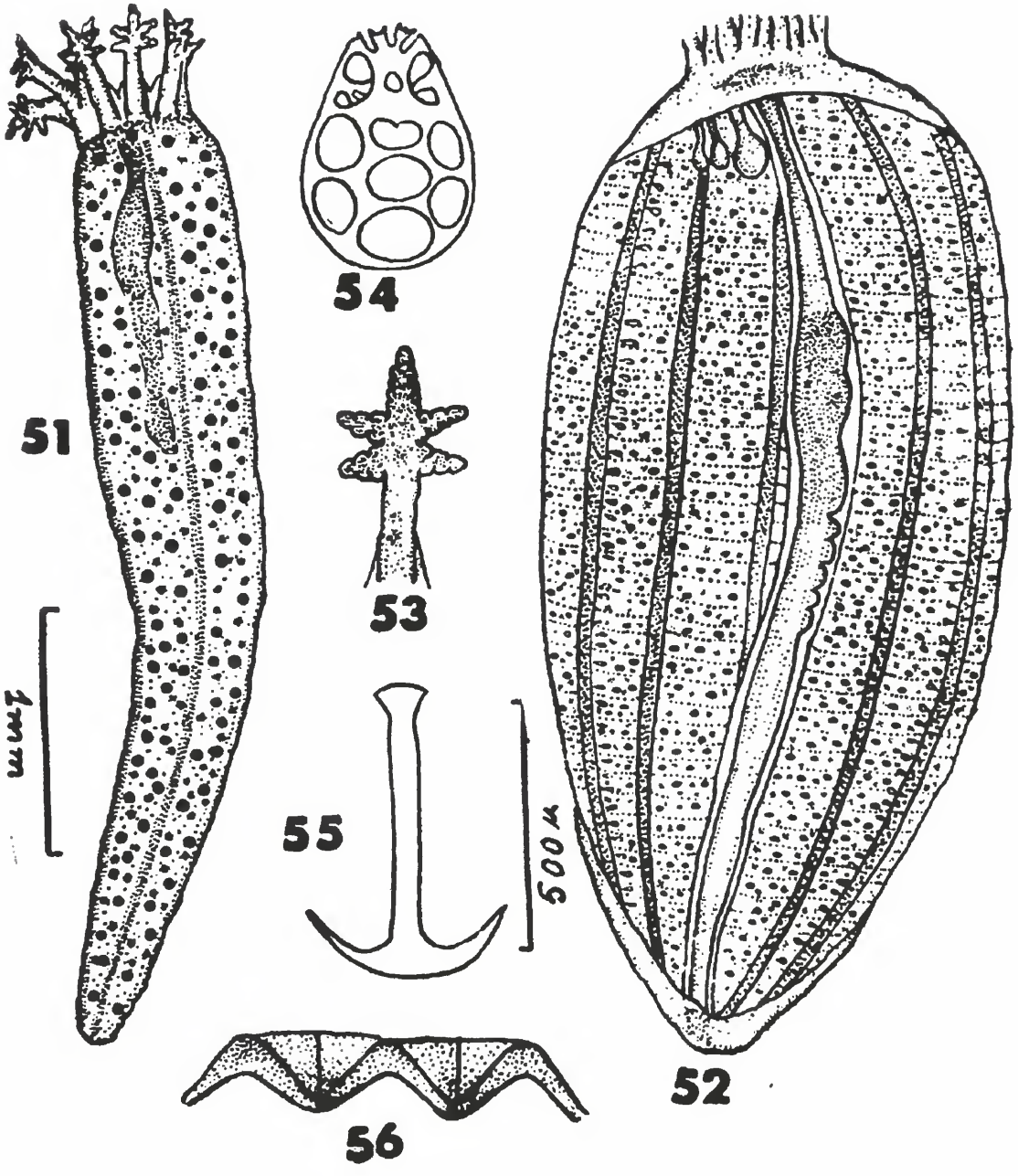
N.º 45 — Animal adulto.
N.º 46 — Roda de carroça.
N.º 47 — Tentáculo digitiforme.
N.º 48 — Dentes do anel calcáreo.
N.º 49 — Chiridota jovem, vista externamente.
N.º 50 — Chiridota jovem dissecada.



ESTAMPA VI

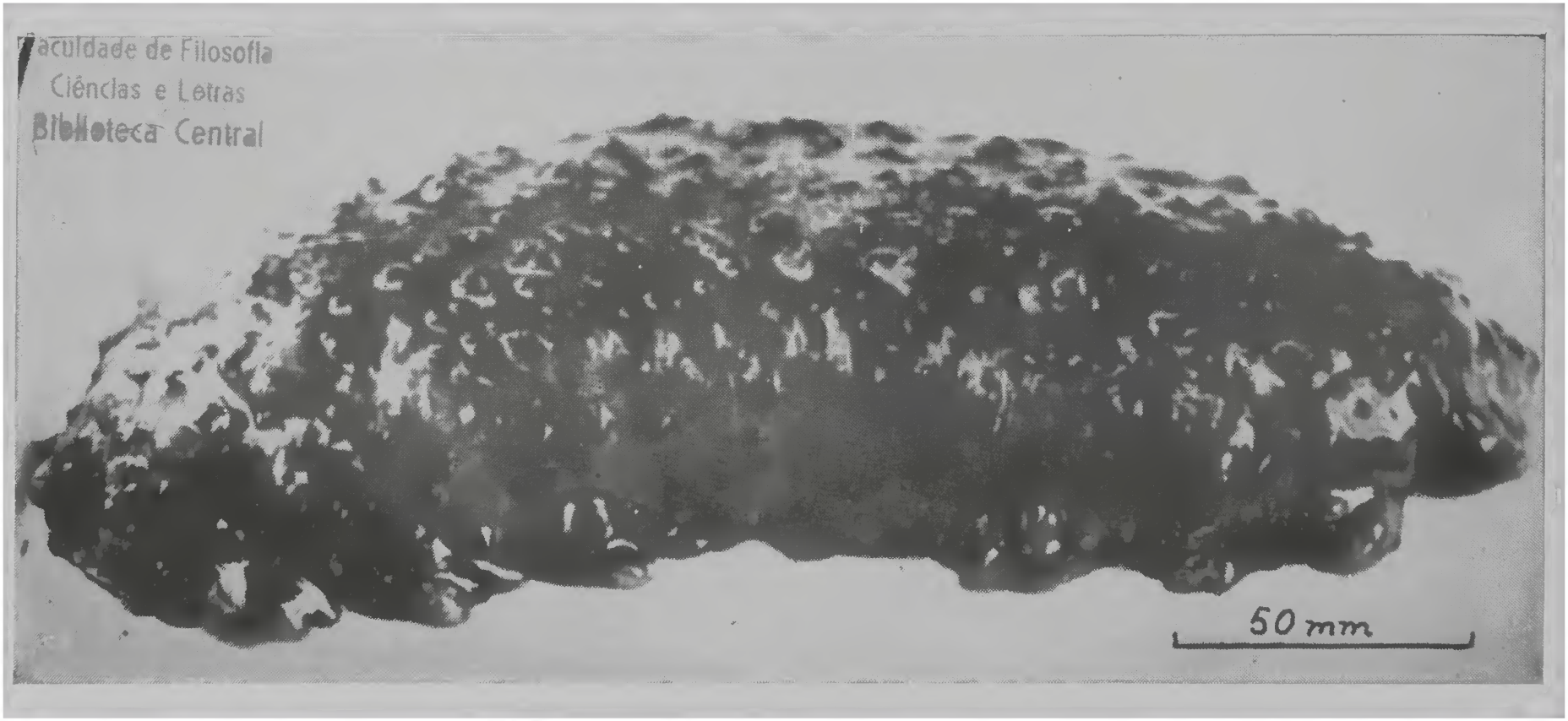
Synaptula secreta, sp. nov.

N.º 51 — Animal adulto.
N.º 52 — Animal aberto mostrando o intestino, as vesículas de Poli e os mm longitudinais simples.
N.º 53 — Tentáculo digitiforme.
N.º 54 — Placa da âncora.
N.º 55 — Âncora.
N.º 56 — Dentes do anel calcáreo.



ESTAMPA VII

N.º 57 — *Stichopus badionotus* Selenka; vista da face dorsal (Foto-Exakta, Biotar 1:2, f. 58 mm).



THE PHARMACOLOGY OF THE INSECT HEART

I. The action of Adrenalin and Acetylcholine on the heart of the Water-bug (*Lethoceros*)

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(Dept. of General and Animal Physiology,
University of São Paulo, Brazil).

INTRODUCTION

The present state of the pharmacology of the Insect heart is unsatisfactory, as BEARD (1953, p. 263) recently pointed out. The problem, according to this author, has been investigated in the intact or in the isolated heart and drugs have been tested at randon or without respect to their modes of action on other systems and there is no reason to believe that the response to drugs is the same in all Insects. Yet, he complains, comparative studies are few and generalizations accordingly cannot be safely made. What is known is the result of two approaches, one with the purpose of explaining normal heart action through the use of drugs having a known action on other animals, the other involving the use of the heart and circulatory responses as a criterion of mode of action of compounds having insecticidal value.

During the course of the class-room work in our Department, with the purpose of showing the heart beat of an Insect, a large water-bug of the genus *Lethoceros* was once dissected and proved to be an excellent material for that kind of study. Even after this first and perhaps not too careful exposure of the heart, the organ immediately resumed its beating and in such a way that I was soon led to record *in situ* the heart beat with the procedure commonly used in the case of the frog heart. The recording was in all respect satisfactory, and therefore the heart of the water bug was chosen for a series of studies of the response of the organ to drugs. In this first paper, the results obtained with adrenalin and

acetylcholine, after preliminary observations on the pH and the saline, are reported.

The action of Adrenalin upon the insect heart was studied by DAVENPORT (1949) in *Stenopelmatus* and he reported that the drug at 10^{-6} retards and at higher concentrations arrests the organ. KRIJGSMANN & KRIJGSMANN (1950), on the contrary, found out that adrenalin stimulates the isolated heart of *Periplaneta*. Acetylcholine was found to accelerate the heart rate of *Melanoplus* (HAMILTON 1939), *Blatta* (PROSSER 1942), *Apis* (PROSSER, l. ci.,), *Stenopelmatus* (DAVENPORT, l. c.) and *Periplaneta* (KRIJGSMANN & KRIJGSMANN, l. c.). HAMILTON (l. c.) in *Melanoplus* observed immediate stimulation with acetylcholine, but also irregularities due to the development of slow rhythmic contractions of the allary muscles. Isolated segments of the heart, however, showed similar types of response and this would indicate that ACh does not act upon a single localized center. DAVENPORT (l. c.) in *Stenopelmatus* found that sensitive heart preparations can be stimulated even with 10^{-6}ACh and that prior treatment with physostigmine makes less sensitive preparations also sensitive to 10^{-6}ACh. Higher concentrations produced a marked acceleration in rate, increased the tonicity and induced a transitory systolic tetany. *ACh* would also restore some activity in fatigued or depressed hearts. Other choline derivatives have also been tested. Acetyl-beta-methylcholine, although less effective than ACh, also stimulates the heart of *Stenopelmatus*. Carbaminoylcholine may induce a systolic tetany similar to that caused by ACh. In view of some cholinesterasic activity of *Melanoplus* heart extracts (MEANS 1942) and of the fact that physostigmine potentiates the action of ACh in *Stenopelmatus*, DAVENPORT (l. c.) suggested that the heart action in insects might represent a cholinergic system.

MATERIAL AND METHODS

Mostly large representatives of the genus *Lethoceros*, measuring from 6 to 9 cm in length were employed in the experiments. In some cases specimens belonging to the genus *Belostoma* were also used. The animals, captured in the surroundings of S. Paulo, were

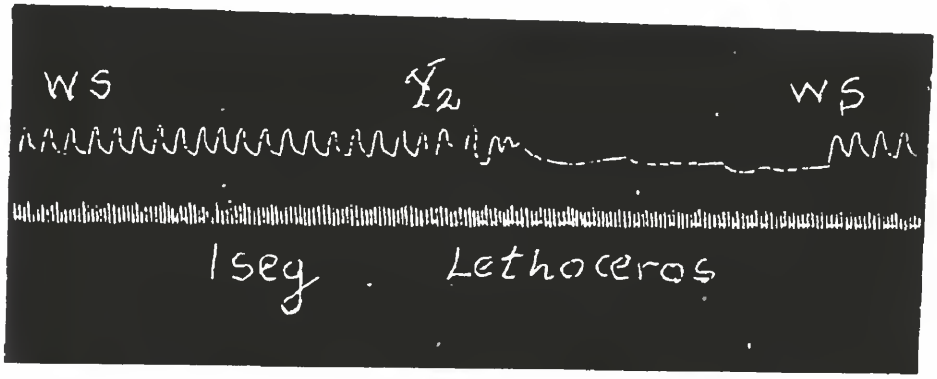
kept in the laboratory in large crystallizers containing pond or simply tap water. In the experiments, the first step consisted in severing the sting and legs at their basis, after previously sectioning the nerve cord in the region between the head and the thorax, behind the subesophagical ganglion. An incision followed separating the abdominal sternites from the tergites. The animal was then pinned down on its backs to a wax tray and the strip of sternum, digestive tract and gonad were carefully lifted away, exposing the dorsal vessel. The preparation from then on was continuously bathed with fresh saline, the wax tray being clamped at an angle so that the perfusion fluid flowed gently over the heart in a posteroanterior direction. Only those preparations which after perfusing with saline for some minutes exhibited a regular heart beat were used in the experiments. In the case of *Lethoceros,* to record the beatings a delicate hook to which a long thread of woman's hair was attached was carefully cramped on the wall of the dorsal vessel and the opposite end of the thread connected with a long (ca. 20 cm) and light aluminium lever. This finally inscribed the beatings on a smoked drum of a kymograph. In the case of *Belostoma,* much smaller in size, the heart rate was checked visually with a stopwatch. Drugs were administered directly to the heart from a pipette, after stopping the continuous flow of saline, and in such a quantity that the remaining traces of plain saline surrounding the heart were completely flushed away and replaced by the drug solution. Acetylcholine chloride from Roche Products Co. and adrenalin chloride from Parke, Davis and Co. were used. The former was dissolved in saline from the solid state and the latter diluted from 10^{-3} aqueous solution contained in the ampules.

RESULTS

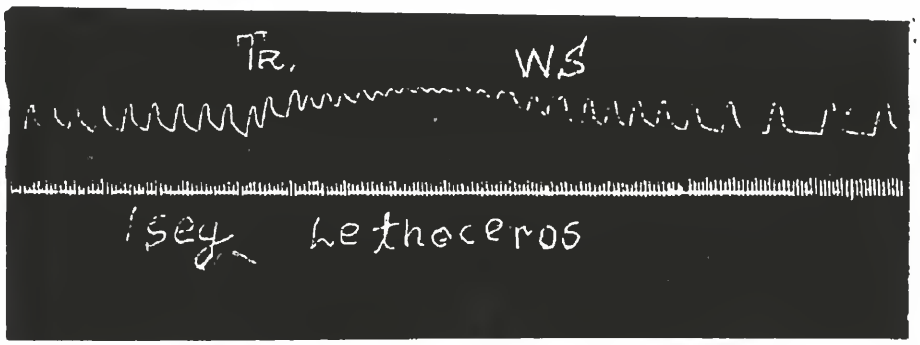
a. *The saline and the pH.* The physiological saline according to WILDER AND SMITH (1938) was used. Its composition is the following: NaCl, 5.5 gm; KCl, 0.14 gm; $CaCl_2$, 0.12 gm and distiled water to a liter, with a pH of 5.5 Acetylcholine dissolved in this unbuffered saline increased the pH, which at 10^{-7} was 5.9, at 10^{-6} 6.2, at 10^{-5} 6.2 and at 10^{-4} 6.3. Adrenalin, on the contrary, decreased the pH, which at 10^{-4} was 3.7, but regularly

increased with further dilutions. In some experiments, the pH of WILDER AND SMITH's saline was adjusted to 7.3 with M/2 Na_2HPO_4, but, as a rule, unbuffered saline was used, since it proved to be more efficacious in restoring the beat of hearts arrested in consequence of traumas or after drug test which caused depression or arrest of the organ. Tracing n. 5 of fig. 3 shows the ability of unbuffered WILDER AND SMITH's saline in restoring the heart beat after severe administration of ACh. Since the pH of this unbeffered saline is 5.5, the suggestion is made that the optimal pH for *Lethoceros* heart is on the acid side of the neutral point.

Another insect saline which was used by YEAGER (1939) in the roach heart and blood and composed of: NaCl, 10.93 gm; KCl, 1.57 gm; $CaCl_2$, 0.85 gm and $MgCl_2$, 0.17 gm, was also tested. This saline caused an immediate stop of the heart beat, which was promptly washed out with WILDER AND SMITH's saline. Even when deprived of $MgCl_2$, the YEAGER's saline could not be used as shown in the tracing 1 of fig. 1. Tracing 2 of fig. 1 shows the action of a saline used in the heart of the Brazilian fresh-



Tracing 1: The effect of YEAGERS's saline deprived of Mg(Y_2) on the heart of the waterbug previously bathed with WILDER & SMITH's saline (WS).



Tracing 2: The effect of a saline (for the Brazilian fresh water crab *Trichodactylus*, Tr) with less Na than WILDER & SMITH's on the heart of the water bug.

water crab *Trichodactylus* (VALENTE, in the press), with a low concentration of NaCl.

b. *The action of adrenalin*. Fig. 2 shows four tracings recorded *in situ* of the heart of *Lethoceros* under the action of different concentrations of adrenalin. It can be clearly seen that with 10^{-7} and 10^{-6} there is stimulation of the heart beat, the frequency principally being increased. With 10^{-5} a tendency to systolic tetany appears and with 10^{-4} this systolic tetany becomes almost complete.

Similar results were obtained with *Belostoma*, the pronounced tendency to systolic tetany being always present with 10^{-5} and 10^{-4} Ad.

c. *The action of acetylcholine*. Fig. 3 shows five tracings recorded *in situ* of the heart beat of *Lethoceros* under the action of acetylcholine solutions from 10^{-6} to 10^{-2}. The tracings indicate that with 10^{-6} there were no changes in frequency, although the amplitude seems to be slightly increased. This increase in amplitude, however, can be considered as a mere hydrostatic effect, since there was an interval between the removal of plain saline and administration of the drug, during which the organ remained wet, but not under liquid pressure. This hydrostatic pressure can clearly be seen in the displacing of the base line of the tracings. With 10^{-5}, 10^{-4} and 10^{-3} the situation was not significantly different and one could hardly speak of essential modifications in the frequency. 10^{-2} ACh was tested to find out just how far could the water bug heart remain indifferent to the drug. An immediate diastolic block was then observed, which was, however, promptly reversible on washing with saline.

The tracings of the figures refer to experiments performed with unbuffered saline. In those using buffered saline, similar results were obtained. As to *Belostoma*, the results were not so uniform, in a few cases a little stimulatory effect of ACh seeming to be present and in a few others depressory effects being observed. As a rule, however, no effect at all was observed.

d. *The cholinesterase activity of the water bug heart*. The cholinesterase activity of extracts of *Belostoma*'s and *Lethoceros*' hearts was checked according to the technique of AMMON (1934).

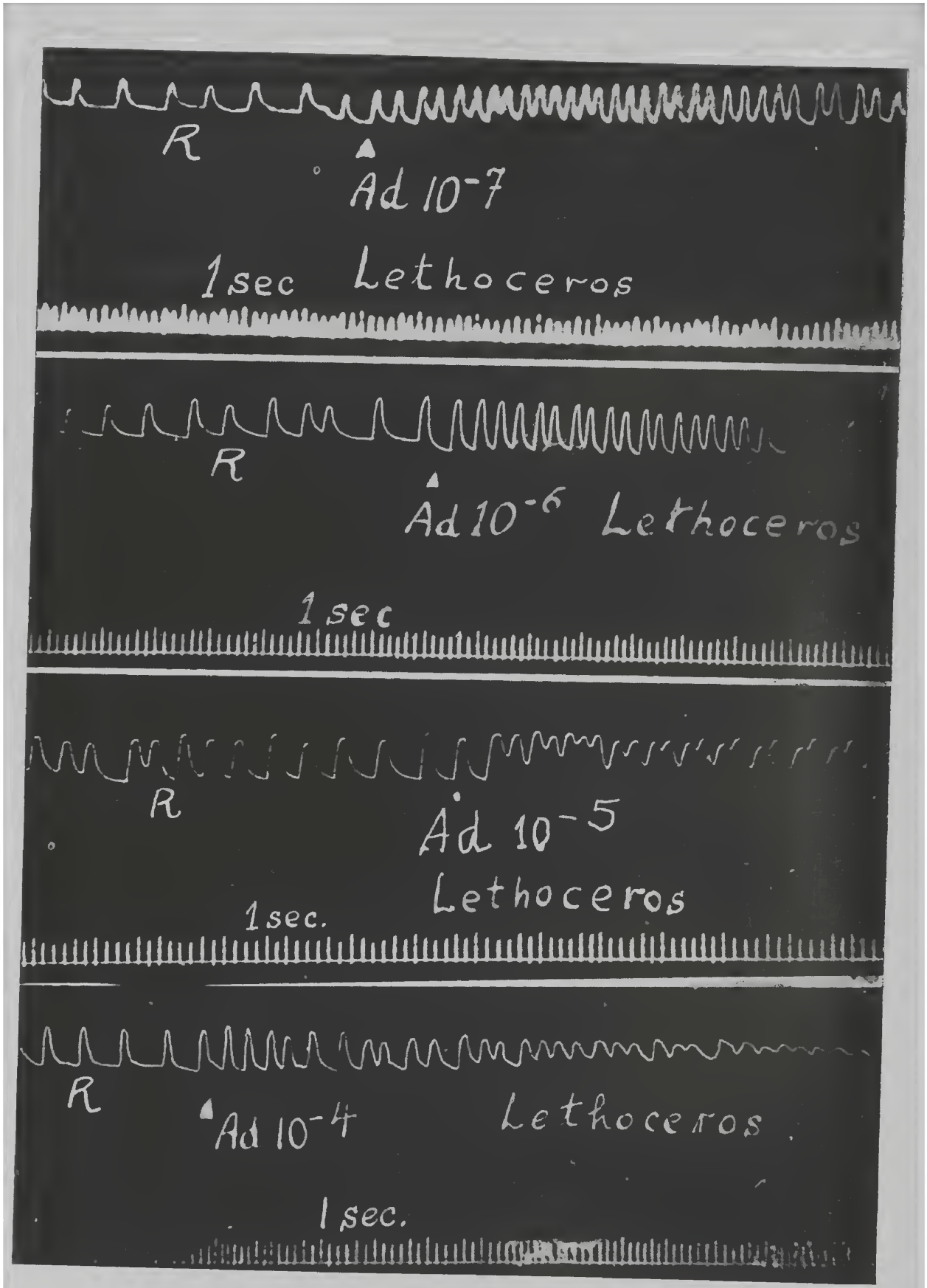

R
Ad 10-7
1 sec Lethoceros
R
Ad 10-6 Lethoceros
1 sec
R
Ad 10-5
Lethoceros
1 sec.
R
Ad 10-4 Lethoceros
1 sec.

Figura 2

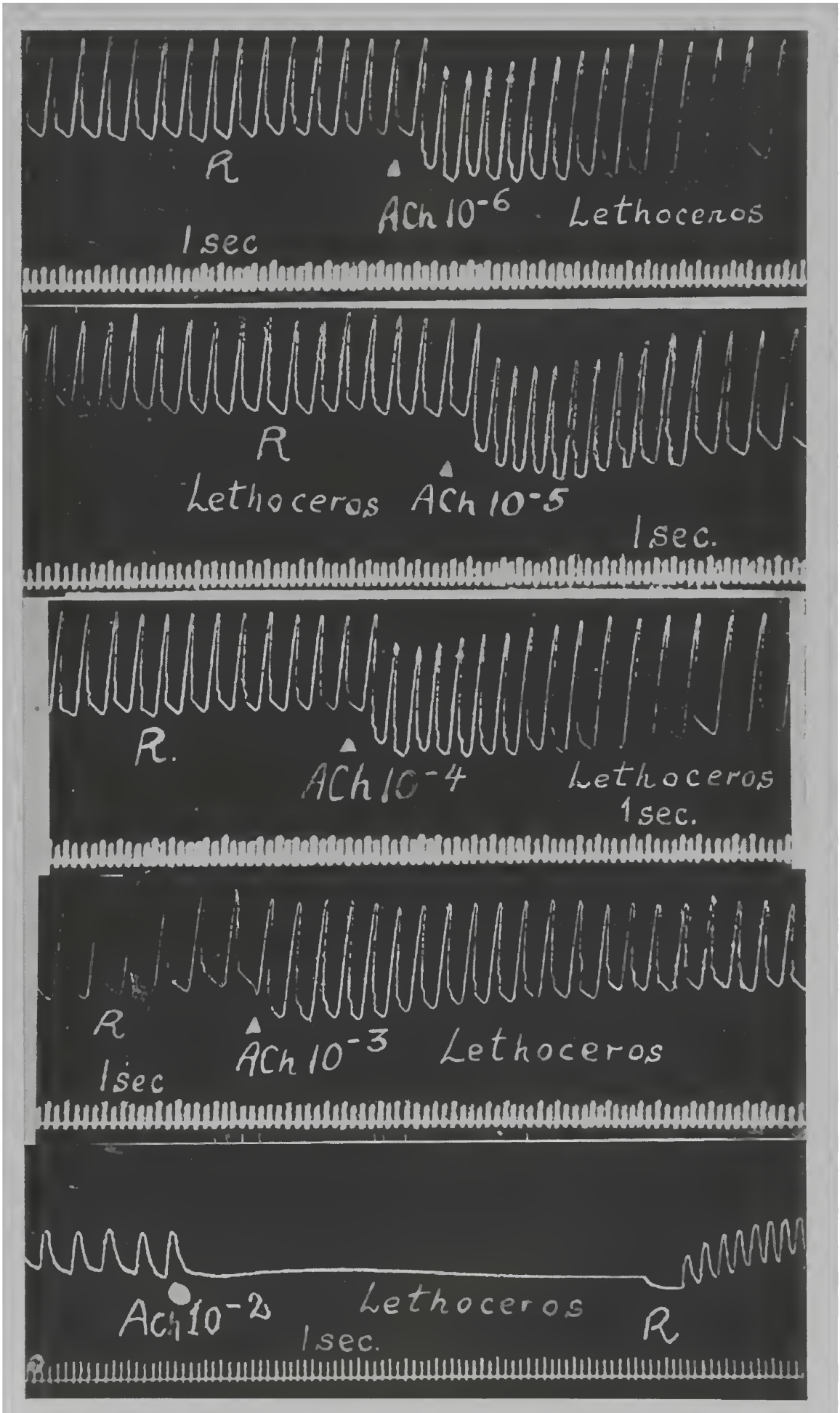

R
ACh 10-6
Lethoceros
1 sec
R
Lethoceros
ACh 10-5
1 sec.
R.
ACh 10-4
Lethoceros
1 sec.
R
1 sec
ACh 10-3
Lethoceros
ACh 10-2
Lethoceros
1 sec.
R

Figura 3

Extracts of 0.5 mg of heart in WILDER & SMITH's saline buffered with bicarbonate were put against 0.1 mg acetylcholine. The heart tissue was obtained by severing the allary muscles under the binocular with an iridectomic scissor at their insertion points. In no case decomposition of acetylcholine could be detected.

DISCUSSION

a. Although it was not the purpose of this work the study of the action of ions on the water bug's heart, some preliminary conclusions can be drawn from the results obtained with WILDER AND SMITH's and YEAGER's salines. These salines differ (see table I) significantly in Na/K and (Na + K)/Ca ratios and in the fact that the latter has $MgCl_2$ whereas the former has not. YEAGER's saline arrested immediately the heart of both *Belostoma* and *Lethoceros*. In DAVENPORT's experiment (l. c.), neither LEVY's, nor MALOEUF's, nor YEAGER AND HAGER's (1934 apud DAVENPORT l. c., p. 23) saline, which I presume has a composition similar to that used by YEAGER AND GAHAN (1937) were able to maintain the heart beat of *Stenopelmatus*. Frog saline, which except for the presence of bicarbonate, is qualitatively and quantitatively similar to WILDER AND SMITH's saline (also a modified Ringer Solution) was then successfully used.

Table I

NaCl, KCl and $CaCl_2$ contents of some salines used in insect heart studies.

	gm NaCl	KCl	$CaCl_2$	water	Na/K	(Na+K)/Ca
Levy 1928	9.00	0.70	0.46	1 l.	12.8	47.0
Maloeuf 1935	9.00	0.20	0.20	1 l.	45.0	47.5
Yeager & Gahan 1937	9.82	0.77	0.50	up to 1 l.	7.0	14.7
Wilder & Smith 1938	5.00	0.14	0.12	up to 1 l.	33.9	47.0
Yeager 1939	10.93	1.57	0.85	up to 1 l.	12.6	21.2
Davenport 1949	6.70	0.15	0.12	up to 1 l.	44.7	45.7

The effects of Na, K and Ca on the insect heart were studied by BERGERARD (1947) in *Gryllus* and DREUX (1950) in *Galleria*. Solutions with Na/K ratios less than 8 stop the heart in

systole. Higher ratios increase the rate of beat but decrease the amplitude. Solution with (Na + K)/Ca ratios of less than 3 cause diastolic arrest, higher ratios retard the rhythm and decrease the amplitude. As to magnesium, FISZER (1950 a and b) reported that when it replaces calcium in a perfusing saline the heart of *Gryllus* is arrested in diastole, but that there is neither synergism nor antagonism between these two ions. Rather, their actions are distinct. There is, however, antagonism between magnesium and potassium. Magnesium is found in insect hemolymph in higher concentration than in man and often (LEVENBOOK 1950 in *Gastrophilus*, FLORKIN 1943 apud BUCK 1953 p. 160 in *Hydrophilus* and BIALASZEWICZ & LANDAU 1938 apud Buck, l. c., p. 160 in *Bombyx*) in strikingly high concentration. FLORKIN (1949) pointed out that Mg is much higher in proportion to the other cations than in most other animals except marine invertebrates and METCALF (1935) suggested that it derives mainly from chlorophyll. The concentration of magnesium in the blood of insect is high enough to induce anesthesia in most non-marine animals. LEVENBOOK (1949 apud BUCK, l. c., p. 160) injected magnesium into the body of adult *Locusta* in a concentration approximating that already present in the blood. Notwithstanding, it rapidly produced a cataleptic condition. After subsequent injection of calcium, which is known to abolish magnesium anesthesia in other animals, the insects recovered quickly. Injection of calcium alone, however, was fatal. LEVENBOOK tentatively concluded that free magnesium as well as free calcium are toxic and that probably they do not occur as such in the insect blood, but bound to protein. On the basis of these results, the failure of LEVY's, of YEAGER & HAGER's salines in maintaining the heart beat of *Stenopelmatus* might be explained on account of relatively low Na/K ratios and perhaps excessively high concentrations of NaCl, KCl and $CaCl_2$ as compared with WILDER AND SMITH's or frog saline (KOZHANTOCHIKOV 1932 apud BEARD, l. c., p. 269 obtained in *Blatta* a systolic standstill with hypertonic Ringer solutions). The failure of YEAGER's saline, on the other hand, in maintaining the heart beat in the water bug can be attributed to the presence of Mg ions, and the low Na/K ratio.

The fact that the optimal pH for the water bug heart seems to be on the acid side of the neutral point can not be considered as astonishing, since it is well known that eight five per cent of the values for the hydrogen-ion concentration of insect blood fall slightly on the acid side of neutrality (BUCK 1953).

b. The responses of both *Belostoma* and *Lethoceros* hearts to adrenalin reveal that the organ is sensitive to the drug. Adrenalin can stimulate the heart beat (10^{-7} and 10^{-6}) or even induce a systolic tetany (10^{-5} and 10^{-4}). These results agree with those obtained by KRIJGSMANN & KRIJGSMANN (l. c.) on the isolated heart of *Periplaneta* where stimulation was also obtained with adrenalin. They are not in agreement with those of DAVENPORT (l. c.) reported for another Orthopteran, *Stenopelmatus,* namely, that the drug at 10^{-6} retards and at higher concentrations arrests the heart in diastole.

c. From the results obtained when 10^{-6} up to 10^{-3} acetylcholine solutions were pipetted upon the water bug heart, it seems that little or even nothing can be said in favor of any particular action of the drug. These results are in complete disagreement with the those of previous authors, which all found stimulatory effects of acetylcholine, as already mentioned. The question naturally arises: Are these differences in effect caused by differences in intrinsic nervous mechanisms of the animals studied? ALEXANDROWICZ (1926) reported the presence of ganglionic cells in the lateral heart nerves of *Blatta*. DAVENPORT (l. c.) found in *Stenopelmatus* ganglia and nerves closely invested to the heart muscle by the surrounding connective tissue. As to *Melanoplus, Apis* and *Periplaneta* in which HAMILTON (l. c.), PROSSER (l. c.) and KRIJGSMANN & KRIJGSMANN (l. c.) respectively found stimulatory effects of acetylcholine, no reference is made in their papers to the presence or absence of ganglia in the heart. From experiment with the water bug *Belostoma flumineum* MALOEUF (l. c.) suggested that in this Hemipteran the heart beat and rhythm are probably independent of posible rhythmic impulses dispatched from ganglionic cells. May be this is also the case in the water bugs used in the present work and that would explain the results

obtained with acetylcholine since according to PROSSER (l. c.) hearts unaffected by this drug are noninnervated.

d. No cholinesterase activity could be detected in extracts of the heart of both *Belostoma* and *Lethoceros,* using the AMMON (l. c.) technique. HAMILTON, using the responses of the frog and turtle hearts to the grasshopper brei also could not detect cholinesterase in this Orthopteran. However, using the Cartesian Diver technique, MEANS (1942) demonstrated that the grasshopper heart extracts has a small (as compared with the nervous and muscular estructures) QCH.E of 0.40. HAMILTON (l. c.) recognized that the absence of cholinesterase in *Melanoplus* rendered difficult a natural function of acetylcholine in that animal's nervous system and, although he obtained acceleration of the heart beat with acetylcholine, he states that "it seems very improbable that it could be of importance in the nerve conduction of this insect", since, among other things, the intact grasshopper is relatively insensitive to acetylcholine. On the basis of the results of MEANS (l. c.), however, and from his own results with physostigmine, DAVENPORT (l. c.) suggested that the heart action in insects might represent a cholinergic system. Although complementary studies are still necessary, the present evidence in the case of the water bug does not seem to support this view.

SUMMARY

1. The action of acetylcholine and adrenalin on the heart of two aquatic Hemipterans (*Belostoma* and *Lethoceros*) was studied.

2. Preliminary tests in order to find out the suitable physiological saline for the experiments indicated that WILDER & SMITH' saline, with Na/K and (NA + K)/Ca ratios similar to those of frog saline used by DAVENPORT in his insect heart studies, was to be adopted. YEAGER's saline which contains Mg Cl_2 stopped the heart of the water bug. A brief discussion of the action of ions on the heart action is given. The fact that the water bug heart beat was better maintained in unbuffered saline (pH ca.5.5) than in saline adjusted to pH: 7.3 with a buffer,

suggests that the pH optimal for the heart action in these insects seems to be on the acid side of the neutral point.

3. The heart beat was recorded *in situ* in the case of the large *Lethoceros* and checked visually with a stopwatch in the case of smaller *Belostoma*. The average heart rate in the former was 24 beats per minute and in the latter 36, at ca. 20°C.

4. Adrenalin acts upon the water bug heart, increasing the frequency in concentrations equal to 10^{-7} and 10^{-6}. At 10^{-5} it induces a systolic tetany, which can be almost complete with 10^{-4}.

5. Acetylcholin produced no modifications on the heart beat of the water bug when used in concentrations from 10^{-6} up to 10^{-3}. When 10^{-2} ACh was tested an immediate diastolic block was observed.

6. No cholinesterase activity was detected when, using the AMMON technique, heart extracts of *Belostoma* and *Lethoceros* were put against acetylcholine solutions.

7. From the lack of action of acetylcholine, from the absence of cholinesterase activity of heart extracts and from the fact that probably in the water bug the heart and rhythm are independent of possible rhythmic impulses from ganglionic cells (MALOEUF in *Belostoma flumineum*) it is here suggested that at least in the Hemipterans studied the heart action does not seem to represent a cholinergic system.

SUMÁRIO

1. Foi estudada a ação da acetilcolina e da adrenalina sôbre o coração de dois insetos hemipteros aquáticos (*Belostoma* e *Lethoceros*), conhecidos por baratas d'gua.

2. Provas preliminares a fim de encontrar o líquido fisiológico mais apropriado para as experiências indicaram que a solução de WILDER e SMITH, cujas relações NA/K e (Na + K)/Ca são semelhantes às do Ringer de Anfíbio usado por DAVENPORT nos seus estudos sôbre o coração dos insetos, era de se adotar. A solução de YEAGER, que contém $MgCl_2$, parou o coração da barata d'água. O fato do coração dêstes insetos se manter

melhor em solução fisiológica não tamponada (pH ca. 5.5) do que na de pH ajustado com tampão a 7.3 sugere que o pH ótimo para a atividade cardíaca parece estar no lado ácido do ponto neutro.

3. O batimento cardíaco foi registrado *in situ* no caso dos grandes exemplares de *Lethoceros* e contados com um cronômetro no caso dos espécimes menores de *Belostoma*. A taxa cardíaca média nos primeiros foi de 24 batimentos por minuto e nos segundos de 36, a 20°C.

4. Adrenalina age sôbre o coração da barata d'água aumentando a freqüência, quando em concentrações de 10^{-7} e 10^{-6}. A 10^{-5} induz tetanía sistólica, que se torna quase completa a 10^{-4}.

5. Acetilcolina não produziu modificações no batimento cardíaco da barata d'água quando usada em concentrações de 10^{-6} até 10^{-3}. Quando 10^{-2} foi experimentada o coração parou imediatamente em diástole.

6. Não se registrou atividade colinesterásica quando, usando-se a técnica de AMMON, colocaram-se extratos do coração de *Belostoma* e *Lethoceros* em contacto com soluções de acetilcolina.

7. Com base na falta de atividade da acetilcolina, na ausência de poder colinesterásico de extratos de coração de barata d'água e finalmente no fato de que provàvelmente nesses hemipteros o batimento e o ritmo cardíaco independem de impulsos rítmicos advindos de células ganglionares (MALOEUF in *Belostoma flumineum*), sugere-se aqui que, pelo menos no caso dos hemipteros estudados, a atividade cardíaca não represente um sistema colinérgico.

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A SURVEY OF HAEM COMPOUNDS IN INVERTEBRATES USING THE BENZIDINE TEST

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INTRODUCTION

In spite of the fact that more than a quarter of a century has already elapsed since KEILIN (1925) published his fundamental work on cytochrome, the occurrence of this pigment in lower organisms has not been widely investigated as HUMPHREY (1947) pointed out. Cytochrome oxidase, on the contrary, has been found to be of frequent occurrence, although there is still much to be done in Invertebrates regarding this enzyme. HUMPHREY (1947, 1948) studied the oyster muscle and there is reason to believe (see WIERSMA 1952) that, in what concerns haem pigments acting on cellular respiration, the situation has not improved much since. Yet, the search for haem compounds in animals is of unquestionable importance and specially interesting in the approaches towards a better understanding of the relation between enzymatic equipment and degree of activity.

Haem compounds acting on cellular respiration are usually detected either by direct spectroscopic observation after crude or more elaborated extraction or by the ability of a tissue extract (or homogenate) to catalyse the air oxidation of reduced cytochrome. Among Invertebrates, however, the amount of such haem compounds can often be to small to be detected by spectroscopical methods and the measurement of oxidase activities by the current techniques may not be considered alone as a full evidence of the presence of haem compounds. The last resort is then the benzidine test. This test, for instance, can afford a further evidence of the presence of the succinic oxidase system in cases where despite

the failure of detecting cytochrome spectroscopically (HUMPHREY 1947) the measurement of the enzymatic activity has shown it to exist. As a matter of fact, the benzidine test should be the first natural step in any study of biological systems possibily involving haem compounds.

The opportunity provided by frequent stays at the new Marine Biological Laboratory of São Sebastião and the vicinity of our laboratory in São Paulo to Institute Butantan led us to survey different marine and terrestrial Invertebrates for haem compounds using the benzidine test. The main scope of the survey was the study of the correlation between the intensity of the response to the test and the degree of activity of the animals. Our thanks are due to the Marine Biological Laboratory and to Institute Butantan for the facilities and material granted and to Drs. W. Bücherl, M. Vannucci and L. R. Tommasi for the classification of some of the animals.

METHODS AND RESULTS

Fresh benzidine was prepared by adding sodium acetate to benzidine hydrochloride. The reaction products were filtered in a Buchner and the deposited benzidine was washed with glacial acetic acid during the filtration. The cake obtained was kept saturated in glacial acetic acid. The test was applied as follows.

Equivalent amounts of total animals or excised parts were ground in a mortar and received 2 ml of benzidine solution plus drops of peroxide (10 vols.). In Arthropods, whenever it was impossible due to the small size of the animals, to excise the soft adhering structures from the chitinous squeleton, the test was applied as did PRENANT (1927) and more recently HANSON (1950), among others, to trace the course of blood vessels. The intensity of the response was arbitrarily classified as negative, scarce, fair and strong, as has been done previously (KEILIN 1925, HUMPHREY 1947), and the assumption was also made that the intensity of the response somewhat reflected the amount of haem compound present.

Table I shows the results obtained and is also an attempt to put together all the available data on the occurrence of haem

compounds in Invertebrates. From the table are excluded data concerning haem compounds which function as blood oxygen carriers or storers, namely, hemoglobin, myoglobin, erythrocruorin and chlorocruorin, because they are already nicely arranged by PROSSER (1952, pp. 291-292, table 48).

As to our own results, the data of table I indicate that:

a) Red sponges exhibited a fair reaction to the test, whereas green and bluish species not only did not respond to the test but also exhibited the striking ability of bleaching the positive reaction given by a solution of pure cytochrome (MENDES & KNAPP 1956).

b) Planctonic medusae gave only scarce responses, whereas Anthozoans showed strong reactions. The Ctenophore did not respond to the test.

c) Terrestrial and marine Isopods exhibited responses from scarce to fair according to the body fragment used. This relation between body part and the degree of the response was still more marked in the case of the two crabs more intensively studied.

d) In the Scorpions and the Spider, the reaction was either negative or scarce for muscles of the movable appendages, but positive when the dorsal abdominal carapace (which included the adhering heart), the fatty abdominal content (spider) or eggs (Scorpion) were used. Scorpion embryos (total, prehatching strages) gave only a fair response.

e) The mantle and body wall muscles of the sea hare (*Aplysia*) strongly responded to the test, whereas total stomach and intestine exhibited a fair response. The oyster foot very definitely responded to the test.

f) The sea cucumber and the sea urchin gave either scarce responses (longitudinal and lantern muscles) or a negative test (intestine, ripe ovary and testis). In the latter case, the material was also able to reverse the positive benzidine test given by pure cytochrome, as in the case of green sponges. The sea lily (Crinoid) neither responded to the nor was able to reverse a positive benzidine test. Ripe ovary and testis of the 9 armed bluish *Luidia* behaved like the same organs of Holothurians and Sea Urchins, bleaching the positive test obtained with cytochrome. On the contrary,

TABLE I

The detection of haem compounds in some marine and terrestrial Invertebrates using the benzidine test.

Phyllum	Group	Species	Organ	Part	Benz. Test	Reversal	Author
Spongiaria	?	red (unclassif.)	total	—	fair	—	Mendes & Knapp 1957
	?	green (")	"	—	negative	+	" "
Coelenterata	Limnomedusae	*Olindias sambaquiensis*	"	—	scarce	—	" "
	Trachymedusae	*Liriope tetraphylla*	"	—	"	—	" "
	Anthozoa	*Bunodactis* sp.	body wall	muscles	strong	—	" "
	"	*Palythoa* sp.	total	—	"	—	" "
	Ctenophora	*Mnemiopsis McGradyi*	"	—	negative	—	" "
Plathyhelminthes	Turbellaria	*Dendrocoela lactea*	?	?	positive (*)	—	Keilin 1925
Annelida	Oligochaeta	*Allolobophora chlorotica*	?	?	"	—	"
		Helodrilus caliginosus	?	?	"	—	"
Aschelminthes	Nematodes	*Ascaris megalocephala*	?	?	"	—	"
		Ascaris suis	?	?	"	—	"
Arthropoda	Xiphosura	*Limulus polyphemus*	heart	total	" (*)	—	Ball & Meyerhof 1940
			claw	yellow mm.	"	—	" "
			squelet.	" "	"	—	" "
			"	white "	negative	—	" "
			blood clot	fraction	positive	—	" "
			eggs	ground	"	—	" "
	Crustacea	*Asellus aquaticus*	?	?	"	—	Keilin 1925
		Oniscus sp.	?	?	"	—	"
		Armadillidium vulgare	thorax	muscles	fair	—	Mendes & Knapp 1957
			"	total	strong	—	" "
			leg	"	"	—	" "
			gut	"	"	—	" "

	Lygia exotica	"	"	scarce	—	"	"
		leg	"	strong	—	"	"
		thorax	muscles	"	—	"	"
	Homarus americanus	heart	total	positive	—	Ball & Meyerhof	1940
		claw	muscles	"	—	"	"
		squelet.	"	"	—	"	"
	Cancer pagurus	?	?	"	—	Keilin	1925
	Grapsoid crab (unclass.)	chela	muscles	scarce	—	Mendes & Knapp	1957
	Callinectes sp.	"	"	"	—	"	"
		walking leg	"		—	"	"
		swimming leg	"	fair	—	"	"
	Uca sp.	chela	muscles	negative	—	"	"
		walking leg	"	scarce	—	"	"
		heart	total	strong	—	"	"
		scaphonathite	muscles	"	—	"	"
Scorpionidea	*Tityus serrulatus*	tail	"	scarce	—	"	"
		chela	"	negative	—	"	"
		cephalothorax	total	strong	—	"	"
	Tityus bahiensis	tail	muscles	scarce	—	"	"
		chela	"	negative	—	"	"
		body cavity	content	fair	—	"	"
		ovary	eggs	strong	—	"	"
		embryos	total	fair	—	"	"
Araneida	*Epeira diademata*	?	?	positive	—	Keilin	1925
	Pamphobeteus roseus	leg	muscles	scarce	—	Mendes & Knapp	1957
		palp	"	"	—	"	"
		cephalothorax	total	strong	—	"	"
		abdomen	content	"	—	"	"
Myriapoda	*Lithobius forficatus*	?	?	positive	—	Keilin	1925
	Geophilus sp.	?	?	"	—	"	

	Insecta	Several Diptera						
		" Hymenoptera						
		" Coleoptera	wing	brown mm.	strong		—	"
		" Lepidoptera	leg	white mm.	scarce		—	"
		" Hemiptera						
		" Orthoptera						
Mollusca	Gastropoda	*Helix*	radula	muscles	positive	(*)	—	Baldwin 1938
			"	"	"	(*)	—	Dhéré & Vegez **
		Helix nemoralis & *aspersa*	?	?	"		—	Keilin 1925
		Limnea peregra & *stagnalis*	?	?	"		—	"
		Busycon canaliculatum	radula	muscles	"	(*)	—	Dakin **
			"	"	"	(*)	—	Mendel & Bradley **
			"	"	"		—	Ball & Meyerhof 1940
			heart	total	"		—	" "
		Aplysia sp.	mantle	muscles	strong		—	Mendes & Knapp 1957
			body wall	"	"		—	" "
			stomach	total	fair		—	" "
			intestine	"	"		—	" "
	Pelecypoda	*Ostrea edulis*	tissues	various	"	(*)	—	McMunn **
		Ostrea sp.	foot	muscles	strong		—	Mendes & Knapp 1957
		Saxcostrea commercialis	foot	muscles	positive	(*)	—	Humphrey 1948
		Venus mercenaria	heart	total	"		—	Ball & Meyerhof 1940
			valves	adductor m.	"		—	" "
	Cephalopoda	*Loligo pealii*	heart	total	"		—	" "
			head	retractor m.	"		—	" "
			neck	" "	"		—	" "
Echinodermata	Holothuroidea	Holothuria grisea	body wall	long. mm.	scarce		—	Mendes & Knapp 1957
			intestine	total	negative		+	" "
			ovary	"	"		+	" "
			testis	"	"		+	" "

	Echinoidea	Arbacia punctulata	testis	sperm	positive	—	Krahl et alt. 1941	
			"	"	positive	—	Ball & Meyerhof 1940	
			ovary	eggs	"	—	"	"
		Echinometra locunter	lantern	muscles	scarce	—	Mendes & Knapp 1957	
			testis	total	negative	+	"	"
			ovary	"	"	+	"	"
	Crinoidea	Tropiometra car.	body	"	"	—	"	"
	Asteroidea	Luidia senegalensis	testis	"	"	+	"	"
			ovary	"	"	+	"	"
		Echinaster	testis	"	strong	—	"	"
			ovary	"	"	—	"	"
			body	fluid	"	—	"	"
			arms	muscles	"	—	"	"
	Ophiuroidea	Ophionereis sp.	disc	total	"	—	"	"
			arms	"	"	—	"	"
Tunicata	Ascidiacea	Molgula manhattensis	intestines	"	scarce	—	Ball & Meyerhof 1940	
		Phallusia mammilata	testis	sperm	positive (*)	—	Califano & Boeri 1950	
			ovary	ripe eggs	"	—	"	"
		Phallusia nigra	blood	fluid	negative	+	Mendes & Knapp 1957	
Acrania	Leptocardia	Branchiostoma platae	body wall	total	strong	—	"	"

* Implication from spectroscopic and enzyme measurements of cytochrome and cytochrome oxidase.

** Authors not mentioned in the literature, cited *apud* Ball & Meyerhof 1940.

the red, 5 armed starfish (*Echinaster*) exhibited ripe ovary and testis and arm muscles with strong benzidine reactions. Finally, the brittle star (Ophiuran) was, among Echinoderms, the one who really gave a remarkable response to the test (as strong as those obtained with cytochrome, Vertebrate blood or Insect wing muscle).

g) The body fluid of the Ascidia was very active in reversing the positive benzidine test obtained with cytochrome. The body wall of the Acranian *Branchiostoma* gave a strong response to the test.

DISCUSSION

Although the main scope of the present survey was to study the relation between the intensity of the response to the benzidine test and the degree of activity of the animal (or part thereof), the results obtained serve also as a starting point to further research of the cellular respiratory enzymes of those animals which definitely gave a positive reaction. For a great majority of the cases gathered in Table I from previous authors this has already been done partially or totally. As a matter of fact, the word "positive" for the benzidine test in cases such as KEILIN's of BALL & MEYERHOF's experiments is a mere implication from spectroscopic and enzyme measurements of cytochrome and cytochrome oxidase, the authors (except for KEILIN's Insect data) actually having not reported results of benzidine tests. The data of Table I suggest the following comments.

Sponges are relatively inactive animals, with no nerve cells. The spindle shaped muscles which bring about the closure of the oscula have been called independent effectors (PARKER 1917) because they combine sensory and motor functions. The fair benzidine test obtained with red species indicates either a small amount of cytochrome (or cytochrome oxidase) or, at least, the hemochromogen precursor mentioned by KEILIN (1929). The negative response given by green sponges does not necessarily mean the absence of haem compounds, since as previously reported (MENDES & KNAPP l. c.), the reversal of the benzidine test obtained with pure cytochrome after addition of ground green sponges

suggests the presence of a compound (possibily analogous to the vanadium chromogen of certain Ascidians) which masks the presence of haem compounds when the test is employed. A further consideration of this point will be made below in the discussion of the results obtained with Holothurians and Ascidians.

The scarce response given by planctonic medusae might be interpreted as a discrepancy between the degree of activity of these lively pulsating organisms and the amount of possibily occurring haem compounds involved in the energy producing cycles. Their strikingly high water content, however, may have influenced the response, the amount of non aqueous material actually used in the tests being much smaller than in other cases. The actinian (*Bunodactis*) and the colonial form *Palythoa* responded according to their well developed muscular system which enables the animals to quickly react to stimulation. The Ctenophore (*Mnemiopsis*) is a feeble swimmer, slowly moving by ciliary action. It also possesses a remarkably high water content. The negative response could then be explained on the basis of these two factors.

The results obtained with Crustaceans call to mind BALL & MEYERHOF's puzzle before the whelk (*Busycon*) and the lobster (*Homarus*): Why should an animal employ hemocyanin for a blood pigment and yet possess muscles rich in myoglobin (*Busycon*) or other haem compounds such as cytochrome and cytochrome oxidase? They did not really attempt to solve this puzzle, nor did HUMPHREY (1. c.) in his oyster's study. BALL & MEYERHOF, however, tried to correlate the occurrence of hemoglobin and hemocyanin with the presence of blood cells. The greater molecular size of hemocyanin would make unnecessary its inclusion in special blood cells. The same would happen to the Invertebrate hemoglobins ("erythrocruorins" ROCHE 1934), of comparable molecular size, which occur in the cell free blood of certain worms. They also stated that hemoglobin, when it does appear as an oxygen carrier, is contained in special blood cells. This view, of course, is supported by the admission that (a) the greater molecular size of extracellular hemoglobins serve to confine the molecules to the circulatory system (PROSSER 1952), (b) hemoglobins in solution can only exist in low concentrations because otherwise it would

cause either a high degree of viscosity (larger molecules) or a high colloidal osmotic pressure (smaller molecules). Besides, within the blood cells haemoglobins would have the proper chemical environment which may be of some funccional significance (BARCROFT 1922). As well known, this admission has contributed to form the controverted opinion that Invertebrate hemoglobins do not function normally as carriers, merely storing oxygen for eventual exposures to low tensions of this gas. We think that a more plausible explanation of BALL & MEYERHOF's puzzle can be offered without appealing to such a controverted argument. The data of Table I for animals possessing hemocyanin, specially our Isopod and Decapod series, indicate that the response to the benzidine test invariably increased when we passed from little active to rhythmically operating structures. This is beautifully shown in the case of the fiddle crab (*Uca*): chela, walking leg, heart and scaphognathite. BALL & MEYERHOF also noticed that in the squid, the most active animal examined, the head and neck retractors have low concentrations of haem compounds as compared with main heart ("the ideal material for demonstrating the cytochrome spectra") and that in the lobster the heart is rich in cytochrome and cytochrome oxidase, whereas the claw and squeleton muscles are poor. Thus, it seems that although using hemocyanin as a blood oxygen carrier, these organisms make use of a fitter intracellular oxidase system (cytochrome and cytochrome oxidase) or oxygen storer (myoglobin) whenever a higher degree of activity is required. We must finally bear in mind that the presence of haem compounds in animals which use hemocyanin as blood oxygen carrier looses much of its puzzling character when consider the widespread occurrence of heme in animals. In fact, it has been utilized in blood or intracellular pigments in a wide variety of unrelated animals regardless of the system transporting oxigen to the tissues (KEILIN 1925, KROGH 1941, PROSSER 1. c.).

The strong response obtained with Spider's or Scorpion's more active organs (such as the abdominal dorsal carapace — which included the heart — and the eggs) and the fair test showed by less active parts (such as the muscles of the movable appendages and late embryos) might be explained along the same line of rea-

soning. The same would also apply to *Aplysia's* data: the strong response of mantle and body wall muscles and the fair test of the stomach and intestine. It is not easy, however, to explain the strong response of the fatty content of the spider.

A nice correlation between activity and presence of haem compounds might have been ascribed to Echinoderms, had we not observed that in most cases where the benzidine test was negative the material was also able to reverse the positive test given by pure cytochrome. In fact, judging by the response alone to the test, one might say that Holothurians, one of JORDAN's (1914) "reflexarme Tiere", and the little active Echinoids and Crinoids exhibited accordingly a negative reaction, whereas the more lively Asteroids and the quite active Ophiuroids strongly reacted to the test. We are not entitled, however, by the Benzidine test alone, to say that Holothurian or sea urchin eggs and sperm do not possess haem compounds, since their occurrence may have been masked during the test by the presence of that compound which is responsible for the observed reversal of the benzidine reaction, as already mentioned. Even in the active bluish starfish *Luidia* the benzidine test was unable to reveal heme derivatives probably in consequence of such a reversing agent. On the other hand, Holothurian longitudinal muscles and sea urchin lantern muscles did respond, although scarcely, to the test and BALL &MEYERHOF (1.c.) reported the presence of cytochrome and cytochrome oxidase in *Arbacia* sperm. They failed, howewer, to detect these haem compounds in the eggs, not even after fertilization (in one experiment with KRAHL). Thus, the situation in the sea urchin seems rather complex. The fact that by methods other than the benzidine test no detectable amount of haem compounds could be found in Arbacia eggs might indicate that if any such compound is really present it cannot be of much functional importance. KRAHL, KELTCH, NEUBECK & GLOWES (1941) also worried about this question and said that "it seems safe to conclude that cytochrome C cannot carry a significant fraction of the oxygen consumption". It remains finally the possibility that the compound responsible for the reversal of the benzidine test could have also acted as an inhibitor in the methods used by BALL & MEYERHOF with *Arbacia* eggs.

The blood of the sessile *Ascidia nigra* strongly bleaches the benzidine test given by a solution of pure cytochrome. Here and in a previous paper (MENDES & KNAPP, l. c.) we have been attempting to correlate this finding (as well as identical results obtained with bluish green sponges and the gonads of Holothurians, sea urchins and of the bluish starfish *Luidia*) with the presence of vanadium, which has already been proved to occur in Ascidians (HENZE 1911) and Holothurians (PHILIPPS 1914). Recently, CALIFANO & BOERI (1950) showed that in the Ascidian *Phallusia mammilata,* the vanadium compound ("hemovanadin") reduces cytochrome both under aerobic and anaerobic conditions. They did not imply, however, that its function is necessarily connected with that of the carrier. The fact that the sperm and ripe eggs of *P. mammilata* exhibit cytochrome c at the spectroscopic analysis raises the interesting question of the compatibility of cytochrome c with such a strong reducing agent within the same organism, which deserves further consideration. BALL & MEYERHOF (l. c.) also noticed the presence of Vanadium in the blood and haem compounds in the tissues of the small Ascidian *Molgula manhattensis*. The fact that they had trouble with the spectroscopic determination of the three cytochromes and the test of the succinodehydrogenase activity may be linked to an interfering action of the vanadium compound.

SUMMARY

1. Several terrestrial and marine Invertebrates were surveyed for haem compounds through the benzidine test, with the main scope of studying the correlation between the intensity of the response to the test and the degree of activity of the animals (or parts therof).

2. Table I shows the results obtained and is also an attempt to put together all the available data on haem compounds in Invertebrates, with the exclusion of those involved in blood transport or tissue storage of oxygen.

3. Using the intensity of the response as a semi-quantitative criterium of the presence of haem compounds a well established correlation with activity could be obtained in cases such as An-

thozoans, Ctenophores, Decapod Crustaceans, red starfishes, Ophiuroids and the Acranian.

4. BALL & MEYERHOF's puzzle concerning the concomitant presence of hemocyanin as a blood carrier and haem compounds as tissue oxygen storer or carrier is discussed in view or the results obtained with Crustaceans, Scorpions, Spiders and Molluscs.

5. The striking ability of some Invertebrate tissues (total green or bluish sponges, the gonads of Holothurians, sea urchins and the bluish starfish *Luidia* and the blood of the Ascidian) to bleach the positive benzidine test given by pure cytochrome (MENDES & KNAPP 1956) is focused in terms of its possible interference with the processes of detecting haem compounds.

SUMÁRIO

1. Diversos invertebrados terrestres e marinhos foram pesquisados pelo teste da benzidina em busca de compostos hêmicos, com a finalidade de estudar a correlação entre a intensidade da resposta e o grau de atividade do animal (ou de suas partes).

2. A Tabela I mostra os resultados obtidos e é também uma tentativa de agrupar os dados disponíveis sôbre os compostos hêmicos em Invertebrados, exclusive os empenhados no transporte sangüíneo ou no armazenamento tissular de oxigênio.

3. Usando-se a intensidade da resposta como critério semiquantitativo da presença de compostos hêmicos, pôde ser obtida uma bem estabelecida correlação com a atividade em casos tais como Antozôos, Ctenóforos, Crustáceos Decápodos, estrêlas do mar vermelhas, Ofiuros e o Acranio.

4. O enigma de BALL K MEYERHOF, relativo à concomitante presença de hemocianina como transportador sangüíneo e compostos hêmicos como armazenadores ou transportadores tissulares de oxigênio, é discutido à vista dos resultados obtidos com crustáceos, escorpiões, aranhas e moluscos.

5. A surpreendente capacidade que alguns tecidos de invertebrados (esponjas verdes ou azuladas totais; gônadas de holoturias, ouriços do mar e da estrêla azulada *Luidia;* sangue da ascídia) possuem de descorar (reverter) o teste da benzidina positivo dado por

citocromo puro é também focalizada em têrmos de sua possível interferência com os processos de evidenciar a existência de compostos hêmicos.

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CONTRIBUTION À L'ÉTUDE DE LA COMPOSITION CHIMIQUE DU SANG DE CERTAINS SÉLACIENS DU BRÉSIL

Préparation de solutions de perfusion

Rubens Salomé Pereira et *Paulo Sawaya*

Au cours d'études sur l'action de l'adrénaline et de la noradrénaline sur la fréquence et sur l'amplitude des battements du coeur de certains sélaciens vivant dans les eaux qui baignent le littoral de l'État de São Paulo — Brésil — (1), nous avons eté amenés à étudier la composition chimique du sang des poissons employés dans les expériences, dans le but de préparer de liquides de perfusion parfaitement adaptés aux animaux étudiés et comme contribution à l'étude biochimique des poissons qui se trouvent dans les eaux brésiliennes.

Les constituants, pour le moment, comportent pour le sang: le fer et les sucres réducteurs; et pour le sérum: les protéines, l'azote non protéique, l'urée, l'azote des amino-acides, le phosphore acido-soluble, le phosphore inorganique, le chlore, le sodium, le potassium, le calcium et le magnésium.

Les animaux etaient recoltés dans la baie de Santos, transportés à l'aquarium, saignés par ponction au coeur. Une partie du sang était mise dans un tube contenant un anticoagulant, l'autre était laissée à la glacière jusqu'à la coagulation. Pour le sucre, le sang était immédiatement traité par le sulfate de cuivre et par le tungstate de sodium.

Techniques chimiques

1.° — Dosage du fer par la méthode à l'acide protocatéquique (Salomé Pereira — 1941a).

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1) U. von Euler et P. Sawaya — Travaux non publiés.

2.° — Dosage du sucre réducteur par désalbumination du sang par le sulfate de cuivre e par le tungstate de sodium, réduction du réactif cuivrique de Harding et Downs (1933) modifié par King et Garner (1947) et détermination photométrique de l'oxyde cuivreux par le réactif arseno-molybdique de Nelson (1944).

3.° — Dosage des protéines par la réaction dite du biuret, après précipitation, dissolution et reprécipitation par l'acide trichloracétique (Fine — 1935; Lieben et Jesserer — 1936; Robinson et Hodgen — 1940, Salomé Pereira — 1944b).

4.° — Dosage de l'azote non protéique par précipitation des protéines par l'acide tungstique, digestion sulfurique, en presence du sélénium, du liquide surnageant après centrifugation énergique et determination photométrique du sel d'ammonium au moyen du réactif de Nessler (Folin et Wu — 1919; Levy — 1936; Campbell et Hanna — 1937).

5.° — Dosage de l'urée suivant Gentzkow (1942).

6.° — Dosage de l'azote des amino-acides par la méthode de Folin (1922) avec les modifications proposées par Danielson (1935), par Sahyun (1939), par Frame et collaborateurs (1943), par Russel (1944).

7.° — Dosage du sodium par photométrie après séparation à l'état d'acétate triple d'uranyle, de magnésium et de sodium (Kahane — 1930; Kahane et Dumont — 1932; Piper — 1932; Hoffman et Osgood — 1938; Herbain — 1949).

8.° — Dosage du potassium par la méthode au cobaltinitrite double d'argent et de potassium (Salomé Pereira — 1945c).

9.° — Dosage du calcium isolé à l'état d'oxalate par la méthode au 2,7-dihydroxynaphtalène (Salomé Pereira — 1951d).

10.° — Dosage du magnésium par la méthode au jaune thiazole (Gillam — 1941; Garner — 1947; Salomé Pereira — 1950e).

11.° — Dosage des chlorures par la méthode à l'iodate d'argent (Sendroy — 1937; Van Slyke et Hiller — 1947).

12.° — Dosage du phosphore inorganique après séparation à l'état de phosphate de calcium, par la méthode céruléo-molybdique de Denigès (Deniès, Chelle et Labat — 1930; Salomé Pereira — 1939f).

La même méthode a eté appliquée à la determination du phosphore acido-soluble après incineration nitroperchlorique.

Les resultats obtenus sont groupés dans le Tableau I et on y trouve aussi la composition de l'eau prise au même endroit où vivaient les poissons examinés.

*

On sait que le milieu intérieur des animaux présente une certaine préssion osmotique qui dépend des protéines du fluid circulant — préssion osmotique colloïdale — et des constituants inorganiques qu'on y trouve — préssion osmotique cristalloïde. Il y a de cas, cependant, où la préssion osmotique cristalloïde est maintenue, dans une très large mesure, par des composés organiques. Un tel phenomène, bien connu, d'ailleurs, se vérifie chez les sélaciens, et le taux d'urée chez le *Rhinobatus percellens* (Walbaum) élasmobranche qu'on trouve au Brésil, est 340 m M/1 environ. En regardant le Tableau I, on voit tout de suite que la teneur en sels minéraux de l'eau de la baie de Santos est beaucoup plus elevée que chez les poissons en étude. Cette hypotonicité est compensée par la rétention d'une grande quantité d'urée, de telle sorte que l'animal est capable de maintenir le gradient osmotique nécéssaire. Puisque l'urée est responsable par 44 pour cent environ de la préssion osmotique du sang des élasmobranches (Duval — 1925) et comme le coeur des élasmobranches de l'eau douce est incapable de battre en l'absence de cette diamide (Baldwin — 1940), nous l'avons incorporée dans les constituants de la solution de perfusion.

Les actions spécifiques des éléments, les rélations entre eux, les antagonismes — ce qui constitue un chapitre très large de la physiologie cellulaire — revèlent qu'il faut établir un rapport convenable entre eux dans les liquides de perfusion, puisque l'irritabilité, la perméabilité, la contractilité et d'autres caractères fonctionnels des tissus requièrent un certain equilibre entre les ions, et comme les éffects d'un élement varient à mesure de la concentration des autres qui se trouvent dans la solution, les rapports ioniques peuvent présenter une signification plus grande que la concentration même des éléments. Il y a de fonctions cellulaires qui varient suivant le rapport *métaux alcalins*: *métaux alcalino-terreux*, ce qui lève

la possibilité d'une correspondance entre le type de fonction d'un organe animal et la composition inorganique de son milieu intérieur (Florkin — 1949).

On sait depuis Bayliss que le liquide Ringer-Lock n'a pas toute son efficacité que si on ajoute une solution capable de lui donner une viscosité égale à celle du sang, et on a employé la gomme arabique à 6 pour cent. Nous avons pensé à substituer à la gomme arabique la polyvinylpyrrolidone, un colloïde synthétique utilisé comme succédané du plasma sanguin (2), dont l'emploi est devenu courant dans la pratique médicale, dissoute dans la solution saline preparée suivant les chiffres du Tableau I.

Comme les animaux mantienent une concentration relativement constante des ions hydrogène dans le sang, comprise la plupart du temps entre pH 7.2 et 7.8, nous avons employé le bicarbonate de sodium pour ajuster le pH de la solution de perfusion.

Dans ce travail préliminaire nous n'avons pas eu l'occasion de suivre les variations saisonières du taux des constituants chimiques du sang des poissons étudiés.

Les solutions de perfusion proposées sont celles qui se trouvent dans les Tableaux II et III.

SUMMARY

In the course of studies on the effect of adrenaline and noradrenaline on the rate and amplitude of the heart beats of certain brasilian selachians (U. von Euler and P. Sawaya — unpublished results) it became necessary to study the composition of the blood of these fishes, both as a contribution to the knowledge of their biochemistry, and as a preliminary step for preparing convenient perfusing solutions for their hearts.

In the present introductory studies, we have performed the following determinations: iron and reducing sugars in the whole blood; and proteins, non protein nitrogen, urea, amino acids nitrogen, acid soluble phosphorus, inorganic phosphates, chlorides, sodium, potassium, calcium and magnesium.

Urea concentration in the blood of selachians is high, this diamide having a considerable influence upon the maintenance of their blood osmotic pressure, and because the heart of fresh water elas-

TABLEAU I

Sang et sérum sanguin de Rhinobatus percellens et de Narcine brasiliensis

Sujet	Sang					sérum								
						Rhinobatus percellens (Walbaum)								
	Fe	Sucre	N-non protéique	N-urée	N-des amino-acides	Protéines	Na	K	Ca	Mg	Cl	P Ácido soluble	Inorganique	Na + K / Ca + Mg
	mg%	mg%	g%	g%	g%	gr	milliéq.	milliéq.	milliéq.	milliéq.	milliéq.	mg%	mg%	
pleine	15,5	30,0	1.120	1.060	20.0	3.25	139.1	7.2	7.7	1.6	150,0	8,4	4,5	15,7
adulte	16,3	26,0	1.050	0.990	22.0	3.91	212.2	8.9	9.0	1.2	214,9	6,5	6,4	21,7
adulte	15,8	24,0	1.150	1.080	21.0	2.92	133.1	13.5	6.4	3.0	115,4	9,5	9,0	15,6
jeune	15,6	25,0	0.942	0.892	21.0	3.34	143.1	11.8	7.6	1.9	137,3	6,5	6,4	13,1
jeune	16,1	40,0	0.990	0.930	21.0	3.42	96.1	16.8	7.0	2.1	94,8	7,2	7,0	12,4
adulte	16,5	24,0	1.060	1.000	18.0	3.20	138.7	10.1	6.0	3.1	135,7	5,8	5,6	16,4
jeune	16,3	127,0	0.990	0.920	23.0	4.00	138.7	14.2	7.1	2.4	134,2	10,2	10,1	16,1
jeune	15,2	25,0	1.000	0.940	30.0	3.64	174.8	20.2	7.5	1.0	166,4	10,9	9,6	22,9
Moyenne	15,9	27,6	1.038	0.977	22.0	3.46	143.2	12.8	7.3	2.0	143,6	8,1	7,3	16,8
						Narcine brasiliensis (Ölfers)								
pleine	—	18.0	0.615	0.585	6.0	2.04	124,8	7.9	9.7	2.0	170.1	9.1	5.0	11.3
adulte	—	16.0	0.630	0.590	10.0	1.95	127,4	6.4	12.6	3.3	159.7	6.9	6.0	8.4
adulte	—	16.0	0.620	0.580	12.0	1.97	149,6	6.8	13.7	3.8	147.5	6.7	5.8	8.9
Moyenne		16.7	0.622	0.585	9.3	1.99	133,9	7.0	12.0	3.0	159.1	7.6	5.6	9.4
Eau de la baie de Santos							354,2	9.8	16.0	75.7		P total - mg 0.04		

(R. S. Pereira — Bol. Fac. Fil., Ciênc. Letr. Univ. S. Paulo — Zool. 9, 69, 1945)

TABLEAU II

Solutions de perfusion pour Rhinobatus percellens (Walbauum)

Solution n.°	Cl Na g.p. 1000 ml	Cl K g.p. 1000 ml	Cl_2Ca g.p. 1000 ml	Cl_2Mg g.p. 1000 ml	Glucose g.p. 1000 ml	Urée g.p. 1000 ml	Polyviyl-pyrrolidon g.p. 1000 r
1	8.37	0.95	0.40	0.094	0.276	—	—
2	8.37	0.95	0.40	0.094	0.276	20.94	—
3	8.37	0.95	0.40	0.094	0.276	20.94	17.30
4	8.37	0.95	0.40	0.094	0.276	20.94	34.60

TABLEAU III

Solutions de perfusion pour Narcine brasiliensis (Olfers)

Solution n.°	Cl Na g.p. 1000 ml	Cl K g.p. 1000 ml	Cl_2Ca g.p. 1000 ml	Cl_2Mg g.p. 1000 ml	Glucose g.p. 1000 ml	Urée g.p. 1000 ml	Polyviyl-pyrrolidone g.p. 1000 ml
1	7.83	0.52	0.67	0.105	0.167	—	—
2	7.83	0.52	0.67	0.105	0.167	12.54	—
3	7.83	0.52	0.67	0.105	0.167	12.54	10.00
4	7.83	0.52	0.67	0.105	0.167	12.54	20.00

mobranchii is incapable of beating in the absence of urea we have added it to the materials composing the proposed perfusing solution.

In order to give to the perfusing solution the required viscosity, we propose to this effect the use of polyvinylpyrrolidone, a synthetic colloidal substance currently employed in medical practice as a substitute for blood plasma.

Sodium bicarbonate was used for buffering the perfusing solutions.

Tables are given.

RESUMO

No decorrer de pesquisas feitas sôbre a ação da adrenalina e da noradrenalina sôbre a freqüência e sôbre a amplitude dos batimentos cardíacos de certos selaceos brasileiros (U. von Euler e P. Sawaya — trabalhos inéditos) fomos levados a estudar a composição química do sangue dêsses animais com o fito de preparar soluções perfusoras convenientes e como contribuição ao estudo da bioquímica dos peixes que vivem no litoral brasileiro.

Determinaram-se, no sangue, o ferro e os açúcares; e no sôro, as proteinas, o azoto não proteico, a uréa, o azoto dos amino-ácidos, o fósforo ácido solúvel, os fosfatos minerais, os cloretos, o sódio, o potássio, o cálcio e o magnésio.

À vista da elevada concentração da uréa encontrada, e do papel que desempenha na manutenção da pressão osmótica do sangue de tais animais, e, ainda, do fato de o coração dos elasmobranquios da água doce ser incapaz de bater na ausência de tal composto, incorporamo-la à solução perfusora.

A fim de dar ao líquido perfusor a viscosidade necessária, propomos a inclusão nele da polivinilpirrolidona, coloide sintético utilizado como sucedâneo do plasma sangüíneo, de emprêgo corrente na prática médica.

Como os animais mantêm de modo relativamente constante, a concentração dos iônios hidrogênio no sangue — entre pH 7.2 e 7.8 em geral — utilizamos o bicarbonato de sódio para ajustar o pH da solução perfusora.

A composição do material analisado e a das soluções de perfusão propostas, encontram-se nos quadros anexos.

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ON A COLLECTION OF BRAZILIAN LAND PLANARIANS

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Dr. Otto Schubart, from the Fisheries Research Station (Estação Experimental de Biologia e Piscicultura) at Piraçununga, State of São Paulo, has kindly forwarded for us to study land planarians he collected during several trips. All the localities are situated in the State of São Paulo, except Ponta Grossa (St. of Paraná) and São José da Barra (St. of Minas Gerais). Nearly all the drawings were made by my wife, Dr. Eudoxia M. Froehlich.

The material comprised the following species:

1. *Geoplana vaginuloides* (Darw.)
2. *G. marginata* Fr. Müll.
3. *G. braunsi* Gr.
4. *G. multicolor* Gr.
5. *G. carinata* Riest.
6. *G. goetschi* Riest.
7. *G. rosea* E. M. Froeh.
8. *G. chita* C. G. Froeh.
9. *G. caapora,* n. sp.
10. *G. poca,* n. sp.
11. *G. schubarti,* n. sp.
12. *G. tapira,* n. sp.
13. *G. toriba,* n. sp.
14. *Issoca potyra,* n. sp.
15. *Bipalium kewense* Mos.

Family **GEOPLANIDAE Stimpson**

Geoplana vaginoloides (Darwin)

Planaria vaginuloides Darwin, 1844, p. 244.

Geoplana vaginuloides Riester, 1938, p. 72; Marcus 1951, p. 54; 1952, p. 76.

Locality: Piraquara, Itanhaen municipality. 1 specimen, 31. VIII. 1941.

The preserved worm measures 38 mm. by 3.8 mm; the mouth is at 21.5 mm., the genital pore at 26.0 mm. from the anterior tip.

The colour pattern is similar to type C of Marcus (1952, pp. 76-77, pl. 23 fig. 136) but the median reddish stripe is broader (about 1 mm. across, just in front of the pharynx), and both the black and the white stripes that follow on each side are narrower (about 0.2 to 0.3 mm. broad each, in the same region). The median stripe begins at 2.5 mm. from the anterior tip. The creeping sole is greyish white.

As seen in the cleared worm, the anatomy agrees with the previous descriptions of the species (Riester, 1939, and Marcus, 1951). The tubular pharynx is ca. 3 mm. long. The penis papilla is 5.3 mm. long.

Geoplana marginata Fr. Müll.

Geoplana marginata Fritz Müller, 1857, p. 24.
Geoplana marginata Graff, 1899, p. 305; Marcus, 1951, p. 56.

Locality: Cananeia, in a wood, 1 specimen. 18.XI.1952.

The preserved worm measures 46 mm. by 3 mm. The mouth is at 23.5 mm., the genital pore at 32.0 mm. from the anterior end. The mesial dorsal stripe is very narrow, the marginal stripes are a little broader than the lateral ones, the colour pattern agreeing, therefore, with that of Marcus, 1951, fig. 151.

The sectioned copulatory apparatus showed that the worm was fully mature. The topography of the copulatory organs (Fig. 1) is similar to that of the worm with inverted copulatory papilla analyzed by Marcus (l. c., fig. 153). There are, however, some differences which may be due to a different state of contraction, to different age, to individual or local variation, or to a combination of these factors. Thus, the seminal vesicle (s) is not straight, but U-shaped; one of the efferent ducts (d) opens at the ental end of the vesicle, the other ca. 0,2 mm. ectally; and the male atrium (a) is relatively shorter, and has fewer but larger folds than in Marcus' material.

Geoplana braunsi Graff

Geoplana braunsi Graff, 1899, p. 309.

Geoplana braunsi Marcus, 1951, p. 60.

Locality: Ibiti, Amparo municipality, 1 specimen at a coffee plantation, 14.IV.1944.

The preserved worm is 128 mm. long by 11 mm. broad. The mouth is at 76 mm., the gonopore at 92 mm. from the anterior end. The colour pattern agrees with Marcus (1951, p. 60). The collar-shaped pharynx is 11.5 mm. long. The copulatory apparatus, as seen in the cleared worm, coincides with Marcus' (l. c.) description.

Geoplana multicolor Graff

Geoplana multicolor Graff, 1899, p. 326.

Geoplana multicolor Marcus, 1951, p. 67.

Localities: Guapiara, 4 specimens, 25.XI.1952. Ponta Grossa, state of Paraná, 1 specimen in a backyard, 8.XII.1952.

All specimens present the same colour pattern as the worm of Marcus, 1951, fig. 291, but the median yellow stripe is narrower, only one sixth to one seventh of the body width. The smallest worm is 15 mm. long by 4 mm. broad, mouth at 8 mm., gonopore at 10.5 mm. from the anterior end; the largest (that from Ponta Grossa) is 30 mm. by 5 mm., mouth at 19, gonopore at 23 mm. from the anterior end. Both these, plus one more from Guapiara were sectioned. The genital apparatus of the two bigger worms were mature and similar to the worm analyzed by Marcus (l. c., pp. 67-8, pl. 28 fig. 177). The smaller worm was incipienthy mature. The female atrium was already filled up by a mass of cells, but the fold that separates the male from the female atria was not yet developed.

Geoplana carinata Riester

Geoplana carinata Riester, 1938, p. 61.

Geoplana carinata Marcus, 1951, p. 70.

Localities: Piraçununga, at the Fisheries Research Station (Estação Experimental de Biologia e Pesca), 1 specimen, 31.VIII.

1945. Monte Serrote, near Juquiá, in a banana plantation, 1 immature specimen, 16.XI.1952. Environs of Itapecerica (town 40 km. SW from São Paulo), 1 specimen, 26.V.1954.

Geoplana goetschi Riester

Geoplana goetschi Riester, 1938, p. 20.

Geoplana fryi var. *bruna* Riester, 1938, p. 69.

Geoplana goetschi Marcus, 1951, p. 72.

Locality: Guapiara (town ca. 200 km. WSW from São Paulo), 1 specimen, 25.XI.1952.

The preserved worm is strongly contracted, being 40 mm. long by 10 mm. broad. The mouth is at 28 mm., the gonopore at 35 mm. from the anterior end. The colour pattern is similar to that of Bresslau's drawing (Riester, 1938, pl. 1 fig. 20), but the lateral stripes are broader and lighter (Fig. 2). The anatomy of the pharynx and the copulatory organs, as seen is the cleared worm, agrees with Riester's (l. c.) and Marcus' (1951) descriptions.

Geoplana rosea E. M. Froeh.

Geoplana rosea E. M. Froehlich, 1955, p. 317.

Locality: Wood near Lagoa Infernão, to the west of the mouth of the Rio Jataí, São Simão municipality (ca. 250 km. N from São Paulo), 1 specimen, 28.III.1955.

Geoplana chita C. G. Froeh.

Geoplana chita C. G. Froehlich 1957 (p. 177).

Already studied in the referred paper.

Geoplana caapora, n. sp.

Locality: Environs of Apiaí, town ca. 250 km. WSW from the city of São Paulo; one specimen, 25.XI.1952.

Preserved (Fig. 3), the worm is 21.0 mm. long by 3.2 mm. broad. The mouth is at 14.0 mm., the gonopore at 16.8 mm. from the anterior end. The body presents subparallel margins, it tapers

rather rapidly to the caudal end, less so to the cephalic end. Both tips are blunt.

The dorsal ground colour (Figs. 3, 5) is light brown (perhaps more yellowish in life). The marginal zone, about a fifth to a fourth of the body width, is dark grey with numerous rounded light spots. These spots are halos around the eyes. At its mesial border, the dark grey zone becomes darker, even black. The broad mesial zone presents, on the ground colour, irregular grey to black patches with rounded indentations, resulting in a mottled appearance. The anterior end (Fig. 4) is also mottled. The ventral side is light grey.

The eyes (Fig. 6) begin marginally and in one row at the anterior tip, but shortly backwards become pluriserial, and at ca. 3.5 mm. from the tip spread on the dorsal surface to about one third of the body width on each side. The dorsal eyes are surrounded by light halos.

Pharynx (Fig. 7) cylindrical; pharynx pocket small; mouth (c) at posterior end of pocket.

Efferent ducts (Fig. 8, d) containing few spermatozoa. Masses of extravasated sperm (sp), issued from burst points of efferent ducts, present in parenchyma near to seminal vesicle (s). Ental part of seminal vesicle paired, with globular dilated lateral portions, each receiveng ventroposteriorly the corresponding efferent duct. Paired part of vesicle, especially globular portions, receives numerous fine-grained eosinophilous and sparse cyanophilous glands. Common part with few glands. Lining epithelium of vesicle columnar, nonciliated, up to 75μ high in globular portions. Ejaculatory duct (e) with narrower lumen than vesicle; nonciliated, and with sparse cyanophilous glands in its ental half; ciliated and with no glands in ectal half. Penis papilla (p) massive, ca. 0.55 mm. long, almost filling up male atrium. Muscularis of papilla rather strong. Basal part of papilla, and atrium are lined by a nonciliated, irregular glandular epithelium. Both eosinophilous and cyanophilous secretion is poured into atrium, the former as balls detached from the epithelium. At tip of papilla opens numerous coarse granular eosinophilous glands, not deeply stained. The ciliated gonopore canal issues from posterior part of male atrium.

Vitellaria mature. Both oviducts (o) contain spermatozoa, indicating recent copulation. Lining epithelium of oviducts containing at free border an accumulation of eosinophilous granules, apparently of epithelial origin. Oviducts begin to rise in front of gonopore. Ascending portion of left oviduct simple, that of right oviduct forks into three branches; two of these branches reunite shortly on, and farther on, with the third branch. Shell glands restricted to ectal portions of oviducts. Oviducts open into a very short female genital canal, a dorso-posterior process of the female atrium. Female atrium globular, opening into male, and filled by a compact mass of cells, looser and mixed with a deeply staining eosinophilous secretion at its central and outer portions. Ventro-anteriorly the female atrium presents a recess, connected also to male atrium, and dilated a little to the left.

The colour pattern of *G. caapora* is distinctive, for no other species of *Geoplana* presents the combination of a grey marginal zone provided with light halos, with a mottled median zone due to an irregular network of grey to black pigment. The colour pattern of *G. fragai* C. G. Froeh. (1955b, p. 197 figs. 4-5) presents a certain similarity, but it has light margins to which the eyes are restricted, no light halos occur in the lateral dark stripes, and in the marbled median zone the pigment occurs chiefly in small longitudinal strips.

The copulatory apparatus of *G. caapora* presents a striking resemblance to that of *G. multicolor* Gr., the chief difference being the absence, in *G. caapora,* of a muscular fold separating the male from the common genital atrium. The pharynges of the two species are also similar. The different colour patterns, however, force their separation.

Geoplana poca, n. sp.

Locality: Piraquara, Itanhaen municipality; one specimen, 30. VIII.1941.

Preserved, the worm is 20 mm. long by 5 mm. broad. The mouth is at 12.5, the gonopore at 14 mm. from the anterior tip. The broadest part of the body is situated at the limit of the third and fourth quarters of its length. From this point it tapers gradually to the

anterior end, rapidly to the posterior one. Ground colour of dorsal side yellow with ferruginous tints. Two lateral black stripes run longitudinally on the back. The inner borders of the stripes are sharply delimited against the ground colour, the outer ones fade towards the margins. Mesially runs a pale dark stripe, formed by elongated dark-gray spots. On the second half of the animal this stripe has darker borders. At the margins of the body there is also a concentration of pigment. The ventral side presents a pale yellowish colour.

The eyes (Fig. 10) begin uniserially at each side of the anterior tip, and shortly backwards become pluriserial and crowded. From ca. 3.5 mm. from the anterior tip on, the eyes spread onto the back, some advancing into the light median zone. The dorsal eyes are surrounded by light halos, bigger in the second third of the body.

Pharynx (Fig. 11) cylindrical; mouth (c) at caudal end of pharynx pocket.

Efferent ducts (Fig. 12, d) full of spermatozoa. Seminal vesicle (s) simple, tubular, looped, extrabulbar, receiving separately the efferent ducts at ental end. Cilia of vesicular lumen 13-15μ long. Unstained glands open into vesicle. Ejaculatory duct (e) narrower than vesicle, ciliated, provided with eosinophilous glands, and slightly winding in the three ental fourths. In ectal fourth there is a contorted portion, then the duct widens into a short glandular, nonciliated portion (u), which receives eosinophilous glands different from those of ental part. This portion is followed by a final one with similar epithelium, but with fewer glands. Penis papilla (p), ca. 0.9 mm. long, nearly fills up genital atrium (ac). Posteroventrally on surface of papilla there is a glandular region, where opens numerous weakly eosinophilous glands (y); rest of papilla coated by an epithelium similar to that of ectal part of ejaculatory duct. Genital atrium lined by a high (70μ, locally), irregular, glandular, columnar epithelium. Subepithelial cyanophilous glands open into atrium. Gonopore (g) near (0.25 mm.) to ventral insertion of penis papilla.

Vitellaria mature. Oviducts (o) rise caudally to gonopore, bend medially and unite into a common oviduct (q) directed ven-

trally. Transverse ectal parts of paired oviducts and common part function as glandular ducts. Common glandular duct open into female atrium, dorso-posteriorly situated in relation to rest of genital atrium. Female atrium separated from this by a fold, which is probably a consequence of pressure exerted by tip of penis papilla against atrial wall.

The colour pattern of *G. poca* is somewhat similar to that of *G. multicolor* Gr., but the lateral black stripes (brown or dark brown in *G. multicolor*) are narrower, and the margins present a concentration of pigment, absent in *G. multicolor*. The eye distribution is also different, *G. poca* presenting the anterior crowding, absent in *G. multicolor*.

By the anatomy of its copulatory organs, *G. poca* belongs to Group C of Brazilian Geoplanas. As regards the size of the penis papilla, it stands between *G. yara* E. M. Froeh. and *G. taxiarcha* Marc. but differs markedly from both in the female part of the copulatory apparatus. The short common glandular duct opening difectly into dorso-posterior part of the genital atrium is distinctive.

Geoplana schubarti, n. sp.

Localities: Piraquara, Itanhaen municipality (Itanhaen city ca. 70 km. south of the city of São Paulo); 2 specimens in a bromelia, 30.VIII.1941; Dr. Schubart col.. Cidade Jardim, a residential quarter in the city of São Paulo; 1 specimen in a small wood, 27.V.1951; Prof. E. Marcus col.

Measures, im mm., of the preserved worms:

Provenience	length	width	mouth	gonopore
Piraquara	30.0	7	16.0	19.0
"	20.0	5	12.5	14.5
São Paulo	26.0	ca. 3	16.0	18.8

The difference in the relative width between the specimen form S. Paulo and those from Piraquara is due to contraction, for the latter were killed with cold alcohol, the former with not fixative (Susa). The specimen from S. Paulo measured, when creeping, 30 x 3 mm.

On the back (Figs. 16, 18) there are three longitudinal black stripes the median one being about half as wide as the broad lateral stripes. The ground colour is orange in the preserved material from Piraquara, light yellow in the specimen from S. Paulo, both living and fixed. It shows itself between the dark stripes and around the margins of the body. The broad creeping sole (Fig. 17), almost as wide as the body, is light grayish-yellow, passing to a darker colour at the anterior and, less intensely, at the posterior end.

At the cephalic end (Fig. 13), the eyes are marginal, in irregular rows. Around the anterior tip the row is discontinuous. At 3 to 4 mm. from the anterior tip, the eyes begin to spread onto the dorsal side. In the smaller worm from Piraquara (Fig. 15), the eyes spread into the light zone between the lateral and the median stripes; in the other two specimens (Fig. 14), the eyes stop at the border of the lateral stripes. Within the dark stripes the eyes are surrounded by small light halos.

Pharynx (Figs. 19, 22) cylindrical. Pharyngeal pocket may present a caudal diverticulum (t) or not (smaller worm from Piraquara).

The two worms from Piraquara are fully mature. The tubular, extrabulbar, contorted seminal vesicle (Fig. 22, s) receives entally and laterally the efferent ducts (d) full of spermatozoa. Into the vesicle opens eosinophilous glands. The ejaculatory duct (e), of smaller calibre than the seminal vesicle, traverses slightly sinuously the penis papilla (p). Both vesicle and ejaculatory duct lined by a cubical or columnar epithelium provided with long cilia (ca. 12μ long in seminal vesicle). Penis papilla large, protruded in both specimens, in the larger one (Fig. 22) broken flush with the ventral surface; in the smaller one about 2 mm. long (Fig. 17, p). On the surface of the penis papilla opens eosinophilous and sparse cyanophilous glands.

Vitellaria fully mature. The oviducts (Fig. 22, o) rise behind gonopore (g). Shell glands (z) open into the ectal parts of the oviducts and into the short common portion (q). Tubular ental part (female genital canal, "vagina") of female atrium directed dorsally and forward. Whole genital atrium lined by a high, nonci-

liated irregular epithelium, with eosinophilous border, and receiving subepithelial cyanophilous glands.

The male part of the genital organs of the specimen from São Paulo (Fig. 20) is mature. The efferent ducts are full of spermatozoa. The large penis papilla (p) is retracted into the genital atrium (ac). The female part is not yet mature. Vitellaria are not developed, and the shell glands (z) are incipient.

By its colour pattern, *G. schubarti* stands isolated among Brazilian Geoplanas. It is similar to *G. aymara* du B. R. Marc. from Peru, but this species has broader latero-marginal stripes that extend to the margin of the body. Besides, in *G. schubarti* the eyes spread much farther on the back than in *G. aymara;* the pharynx is less folded, and the female part of the copulatory apparatus is quite different.

By the anatomy of the copulatory aparatus, *G. schubarti* belongs to Group C of Brazilian Geoplanas, standing near *G. livia,* E. M. Froeh., *G. riesteri* C. G. Froeh. and *G. joia* C. G. Froeh., but the colour pattern is wholly different from any of these species.

Geoplana tapira, n. sp.

Locality: Tapiraí, a small village ca. 100 km. WSW of the city of São Paulo, between Piedade and Juquiá; one specimen in a wood, 15.XI.1952.

The preserved worm (Fig. 21), 38 mm. long by 12 mm. broad, is strongly contracted for being preserved in cold fixative (alcohol). The mouth is at 27, the gonopore at 32 mm. from the anterior tip. The body is broad, with subparallel margins; it narrows rather abruptly at both ends, less so at the anterior one, which ends in a pointed tip.

On the back there is a pair of longitudinal black stripes separated by a median light line. Each stripe is 2-2.5 mm. broad. Towards the ends they narrow and posteriorly stop at a distance from the tip. The ground colour, evident in the median line and at the broad latero-marginal zones, is yellowish-ochre. The ventral side is dirty yellow with a narrow border of the dorsal ground colour; the anterior tip is brown. The sensory border (Sinneskante) is visible to about 7 mm. from the tip.

The eyes (Fig. 23) circle the anterior end in one row, and then become pluriserial, but does not spread on the dorsal surface more than 0.8 mm. on each side.

Pharynx (Fig. 24) cylindrical, long, ca. 5 mm. long from insertion; free border richly folded. Pharynx pocket extends backwards to the vicinity of the seminal vesicle.

Worm incipently mature. Up to five testes in same side of transverse sections. Testes with no ripe spermatozoa. Empty efferent ducts (Fig. 25, d) open separately into seminal vesicle (s), extrabulbar in position, but enveloped by fibres common to penis bulb. Epithelium of vesicle columnar, ciliated, containing ducts of eosinophilous and weakly cyanophilous glands. Ejaculatory duct (e) newly opened, simple, lined by a cubic epithelium with cyanophilous border. Penis papilla (p) massive, ca. 1 mm. long, filling up male atrium; tip of papilla bent to the right. Male atrium (a) separated from female by a constriction.

Vitellaria absent. Oviducts (o) rise in front of gonopore (g) and fuse dorsally into a common oviduct (q) directed backwards, that opens dorsally into middle part of female artium. Shell glands not yet formed. Female atrium filled up by a mass of cells (r), many of them containing eosinophilous granules. Gonopore canal exits from ventro-anterior part of female atrium.

G. bilinearis (Darw.), *G. bilineata* Fuhrm., and *G. theresopolitana* Schirch, known only by external characters, are species with a pair of dark stripes on the back, but are smaller and more slender species than, and the position of the stripes is different from *G. tapira*. *G. gabriellae* du B.-R. Marcus has also two dark dorsal stripes, but the position of the stripes, and the anatomy of the copulation organs are different.

Like several species of *Geoplana* (cf. E. M. Froehlich, 1955, p. 329), *G. tapira* presents a mass of cells of unknown function in the female atrium. Besides this common feature, *G. tapira* is distinct from any of these. Riper worms are needed for judging its relationships within the genus.

Geoplana toriba, n. sp.

Locality: Monte Serrote, near Juquiá (town ca. 130 km. SW of São Paulo); 1 specimen in a banana plantation, 16.XI.1952.

Preserved (Fig. 28), the worm is 40.5 mm. long by 5.0 mm. broad. The mouth is at 24.7; the gonopore, at 28.2 mm. from the cephalic tip. The body is rather flat, with subparallel margins. Both anterior and posterior narrowings rapid, but not abrupt.

On the back there are two pairs of narrow dark stripes, one pair marginal, the other bordering a reddish ochre median zone. Between the marginal and the submedian stripes there is a yellow zone. The submedian pair fuse both anteriorly and posteriorly. Halos of eyes occur in the marginal stripes and in the median zone, those of the latter being larger. The ventral side is light grey. The grey tint is due to a loose net of dark pigment.

Except to about 7 mm. from the anterior tip, where they are marginal (Fig. 29), the eyes spread onto the whole dorsal side (Fig. 30). The pigment cups of the larger anterior eyes have a diametre of ca. 55μ and a length of 75μ.

Pharynx (Fig. 26) cylindrical, contracted, 1.3 mm. long from ventral insertion. Pharynx pocket 2.5 mm. long.

Efferent ducts (Fig. 27, d), containing spermatozoa but not dilated into spermiducal bulbs, open into paired transverse portions of extrabulbar seminal vesicle (s). Seminal vesicle tubular, bent, lined by a cubic ciliated epithelium, and receiving eosinophilous and weakly cyanophilous glands. Ejaculatory duct (e) similar to vesicle, but lacking eosinophilous glands; its bulbar portion and ental papillar portion, looped; rest of papillar portion nearly straight, opening at tip of papilla. Penis papilla (p) 0.7 mm. long. Near the posterior end of the papilla a circular flap encloses partially the tip. Epithelium of penis cubical to columnar, irregular, nonciliated. Male atrium separated from female by a pair of lateral folds. Epithelium of male atrium columnar, irregular, nonciliated, with free border full of eosinophilous granules. On a projecting atrial fold over the penis papilla open numerous cyanophilous glands (y), into the rest of the male atrium, scarce cyanophilous glands.

Vitellaria mature. Oviducts (o) rise very steeply, caudally to gonopore. Shell glands (z) open into ectal half of the ascending

portion of the oviducts, into final transverse portions of the same, and into short, postero-ventrally directed, common glandular duct (q). This duct continuous with female genital canal ("vagina"), the ental tubular part, upward turned, of the female atrium. Female atrium (f) ample, lined by an epithelium similar to that of male atrium, but higher and with a thicker layer of eosinophilous granules at the free border. Genital pore canal issues from anterior part of female atrium.

G. rostrata Gr. has also two pairs of black stripes on the back, but the central pair has a more lateral position than in *G. toriba*, resulting in a broader median zone. This zone is plain yellow, not ochraceous with light halos, and the eyes do not enter into it. Besides, *G. rostrata* has a pair of gray stripes on the ventral surface, absent in *G. toriba*.

By the structure of the copulatory apparatus, *G. toriba* belongs to group C of Brazilian Geoplanas, standing near to *G. pavani* Marc., which presents, however, a much bigger seminal vesicle, and a smaller penis papilla. The colour pattern of these two species are wholly different, *G. pavani* being spotted.

Issoca potyra, n. sp.

Locality: Eldorado (formerly Xiririca), town on the Rio Ribeira (Ribeira River), ca. 190 km. SW from the city of São Paulo, 1 specimen in a coffee plantation, 21.XI.1952.

Preserved (Fig. 31), the worm is 39 mm. long by 5.5 mm. broad. The mouth is at 21.7, the genital pore at 30.2 mm. from the anterior end. From its broadest part, about the middle, the body tapers gradually to both ends. The anterior end is blunt, due to the presence of the glandulo-muscular organ.

On the back there is a pair of black longitudinal stripes, ca. 1 mm. broad each, at the pharynx level. At the same level, the median zone encolosed by them is 0.8 mm. broad. The dorsal epidermis is irregularly spotted with brown. The parenchyma, except for the dark stripes, has no pigment, being of a light yellowish-grey colour. In life the ground colour is probably lighter, for after some time the epidermis (specially the rhabdoids) darkens in the usual preserving fluids (alcohol and formalin). The ventral side is light

yellowish-grey, with a narrow brown border; at the anterior end (Fig. 32) is found the broad horseshoe-shaped glandular surface. The position of the sensory tract (Sinneskante) is similar to that of *I. rezendei* (C. G. Froehlich, 1955a, pl. 8 f. 46).

The eyes circle the anterior tip (Fig. 33) in one row. The foremost eyes are provided with long pigment cups, 50-60μ long by 20-27μ wide; the rest of the eyes present the ordinary short cups with a diametre of 30-45μ. About 4 mm. from the anterior tip, the eyes spread on the dorsal surface (Fig. 34) to the black stripes, where they are surrounded by small light halos.

The structure of the glandulo-muscular organ (Fig. 35) is similar to those of the other species of the genus (C. G. Froehlich, l. c., p. 226, pl. 9).

Pharynx (Fig. 36) cylindrical with dorsal insertion caudally displaced, approaching bell type (Glockenförmig) of Graff. Mouth (c) at beginning of second third of pharynx pocket.

Efferent ducts (Fig. 37, d), full of spermatozoa, open into each side of seminal vesicle (s). Seminal vesicle (Fig. 38) extrabulbar, complex, with numerous interconnections between its parts. Ental two thirds of ejaculatory duct (Fig. 37, e) wide and irregular, ectal third tubular. Both vesicle and ejaculatory duct receive cyanophilous and eosinophilous glands, and are lined by a cubic or low columnar ciliated epithelium. Ejaculatory duct opens on ventral side of short conical penis papilla (p). In neighbourhood of ejaculatory duct opening, papilla lined by a cubical nonciliated epithelium. Rest of penis papilla, as well as male atrium (a), lined by a columnar, nonciliated epithelium provided with an accummulation of erythrophilous granules at the free border. At ectal end of male atrium, especially on the dorsal fold (x) that separates it from female atrium, open numerous cyanophilous glands (w).

Vitallaria fully mature. Oviducts (o) begin to rise in front of gonopore (g). Shell glands (z) open into ectal ascending portion and into transverse final portion of paired oviducts, and into backwards directed common glandular duct (q). This duct opens into ental, slightly dilated portion of female atrium, separated from the rest of the atrium by a constriction. Whole female atrium (f) lined by an epithelium similar to that of male atrium, but receiving

numerous cyanophilous glands. Muscular coat of female atrium distinct from that of male (which is here the penis bulb musculature). Genital pore canal issues from anterior part of female atrium.

Issoca potyra differs from the known species of the genus in having but one pair of dark stripes. It bears a certains likeness to *I. piranga* C. G. Froeh., which has a pair of broad lateral black stripes, and one of narrow marginal ones. As regards the copulatory apparatus, *I. potyra* stands near *I. piranga* too. This species has, however, a simple seminal vesicle, a narrower ejaculatory duct, and a more folded atrium.

Family **BIPALIIDAE** Graff

Bipalium kewense Mos.

Localities: Ibiti, Amparo municipality, 1 specimen, april 1944 São José da Barra, Minas Gerais State, 2 specimens under a rotting log in a grazing field, 1.III.1953.

Resumo.

Neste trabalho são estudadas taxonômicamente planárias terrestres coligidas em diversas excursões pelo Dr. Otto Schubart. O material incluia 15 espécies, das quais 6 novas. Quase todo o material provém do Estado de São Paulo.

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PLATES

PLATE 1

Geoplana marginata Fr. Müll.

Fig. 1 — Copulatory apparatus, combined sagittal sections.

Geoplana goetschi Riest.

Fig. 2 — Colour pattern of dorsal side.

Geoplana caapora, n. sp.

Fig. 3 — Dorsal view of preserved worm.

Fig. 4 — Dorsal view of anterior tip.

Fig. 5 — Dorsal view, about the middle of the body.

Fig. 6 — Distribution of the eyes at the anterior end. The tip is bent to the right.

Fig. 7 — Median section of pharynx.

Fig. 8 — Copulatory apparatus, combined sagittal sections.

a, male atrium; b, penis bulb; c, mouth; d, efferent duct; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, muscularis of pharynx; m, muscle coat of copulatory apparatus; o, oviduct; p, penis papilla; q, common glandular duct; r, mass of cells of female atrium; s, seminal vesicle; sp, mass of spermatozoa; y, eosinophilous glands; z, shell glands.

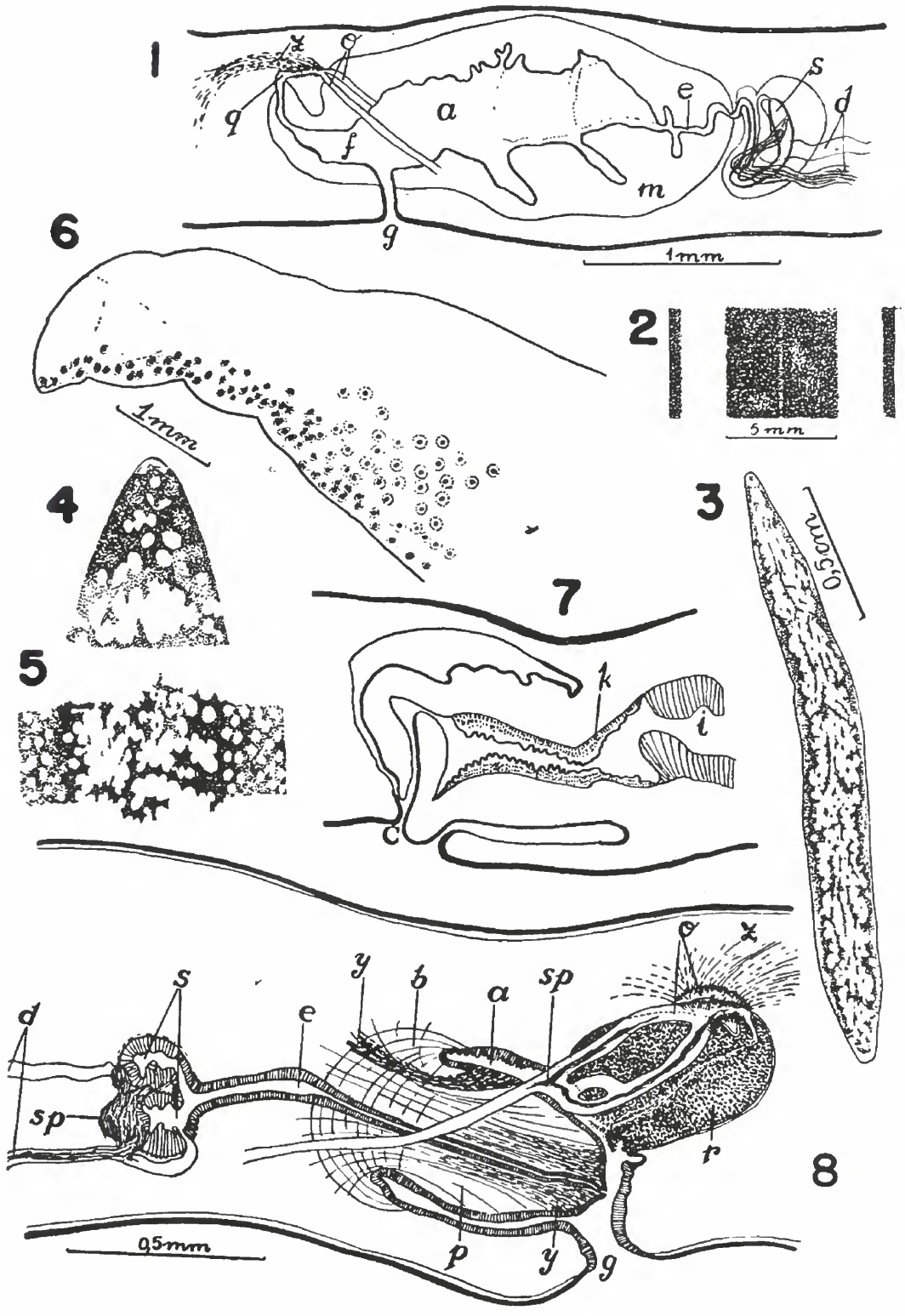

PLATE 2

Geoplana poca, n. sp.

Fig. 9 — Dorsal view of preserved worm.
Fig. 10 — Distribution of the eyes at the anterior portion of the body.
Fig. 11 — Pharynx, median section.
Fig. 12 — Copulatory apparatus, combined sagittal sections.

Geoplana schubarti, n. sp.
(also pl. 3, Figs. 16-20; pl. 4, Fig. 22)

Fig. 13 — Distribution of the eyes at the anterior end (larger specimen from Piraquara).
Fig. 14 — Distribution of the eyes in front of the pharynx (larger specimen from Piraquara).
Fig. 15 — Distribution of the eyes in front of the pharynx (smaller specimen from Piraquara).

ac, common genital atrium; b, penis bulb; c, mouth; d, efferent duct; e, ejaculatory duct; g, gonopore; i, intestine; o, oviduct; p, penis papilla; q, common glandular duct; s, seminal vesicle; u, dilated portion of ejaculatory duct with eosinophilous glands; y, eosinophilous glands; z, shell glands.

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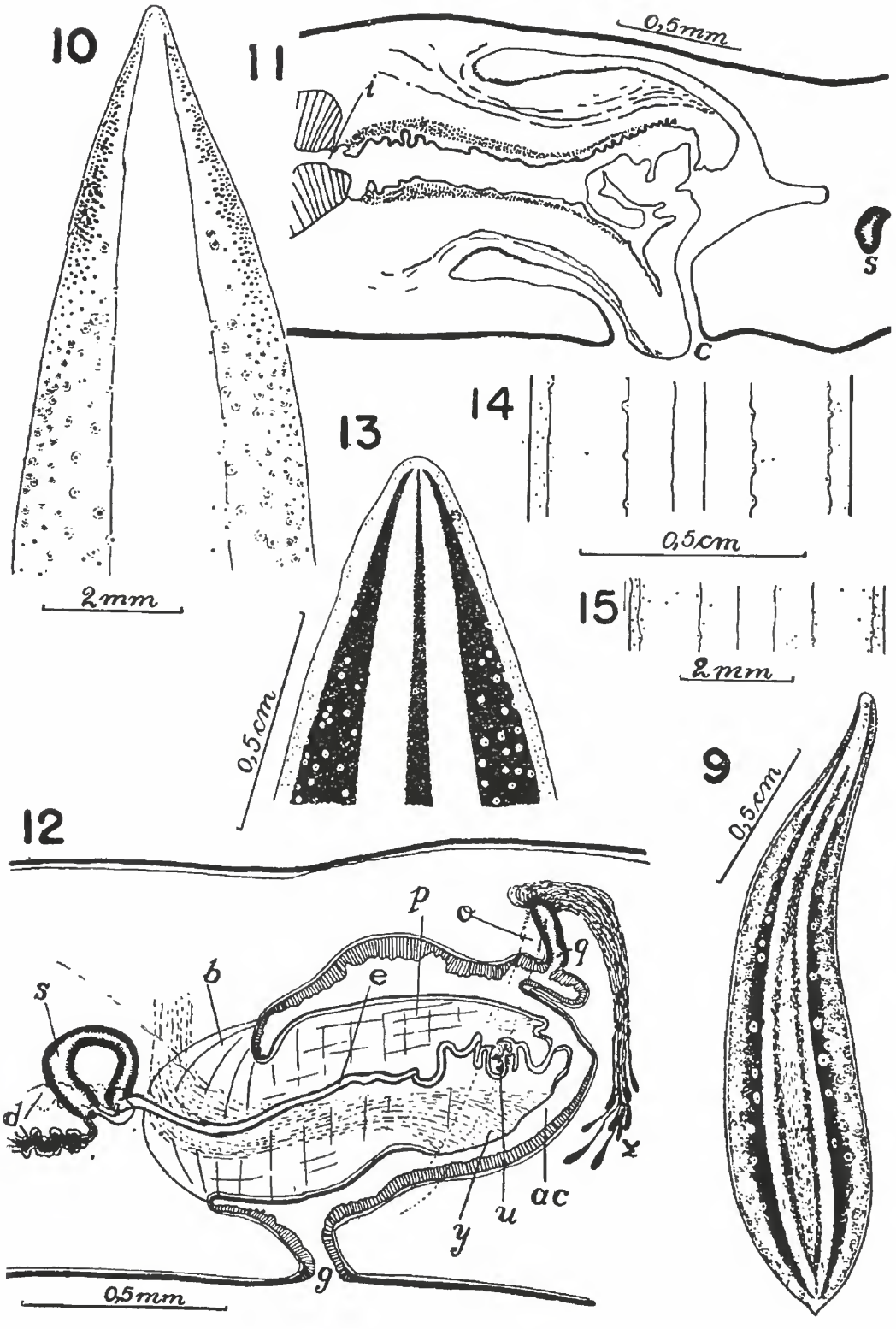

PLATE 3

Geoplana schubarti, n. sp.
(also pl. 2, Figs. 13-15; pl. 4, Fig. 22)

Fig. 16 — Dorsal view (larger worm from Piraquara).
Fig. 17 — Ventral view (smaller worm from Piraquara).
Fig. 18 — Dorsal view (specimen from São Paulo).
Fig. 19 — Median section of the pharynx (specimen from São Paulo).
Fig. 20 — Copulatory apparatus, combined sagittal sections (specimen from São Paulo).

Geoplana tapira, n. sp.
(also pl. 4, Figs. 23-25)

Fig. 21 — Dorsal view of preserved specimen.

ac, common genital atrium; b, penis bulb; c, mouth; d, efferent duct; e, ejaculatory duct; g, gonopore; i, intestine; k, muscularis of pharynx; o, oviduct; p, penis papilla; q, common glandular duct; s, seminal vesicle; sb, sensory border; t, pharynx pocket; v, female genital canal (vagina).

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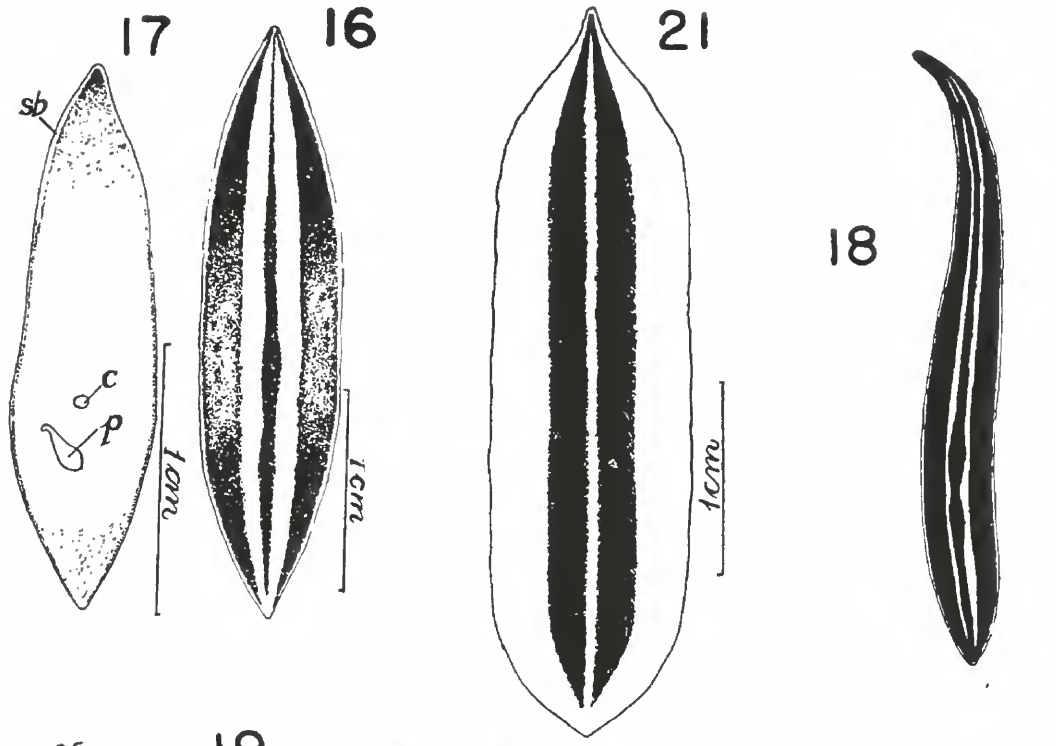
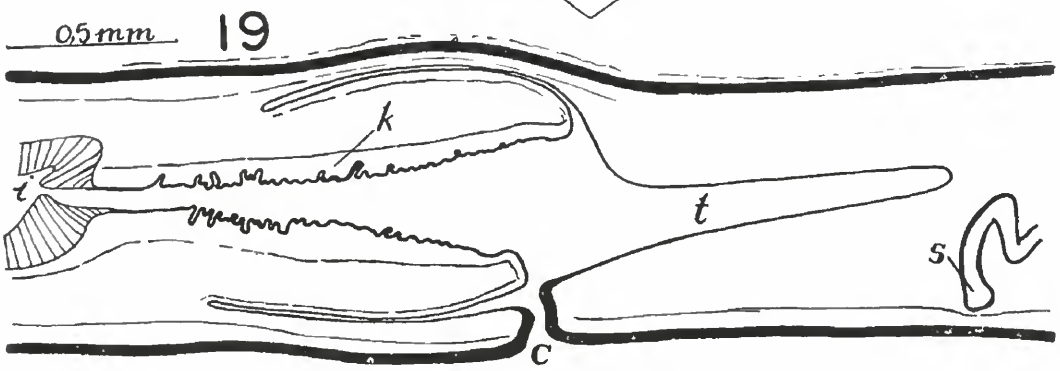




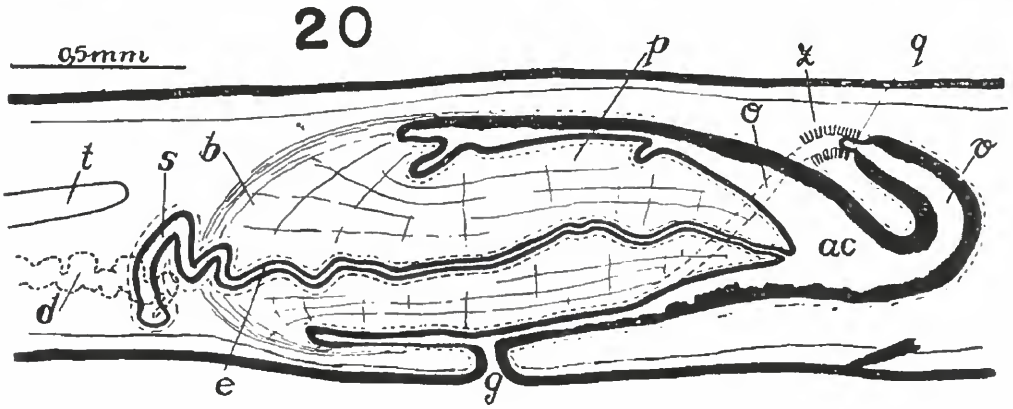


PLATE 4

Geoplana schubarti, n. sp.
(also pl. 2, Figs. 13-15; pl. 3, Figs. 16-20)

Fig. 22 — Pharynx and copulatory apparatus, combined sagittal sections (larger worm from Piraquara).

Geoplana tapira, n. sp.
(also pl. 3, Fig. 21)

Fig. 23 — Dorsal view of anterior portion of the body, showing the distribution of the eyes.

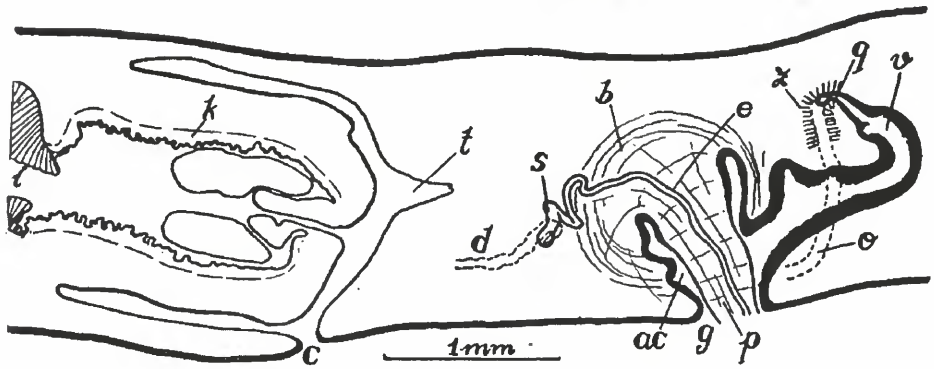
Fig. 24 — Pharynx, median section.

Fig. 25 — Copulatory apparatus, combined sagittal sections.

Geoplana toriba, n. sp.
(also pl. 5, Fig. 27-30)

Fig. 26 — Pharynx, median section.

a, male atrium; ac, common genital atrium; b, penis bulb; c, mouth; d, efferent duct; e, ejaculatory duct; g, gonopore; i, intestine; k, muscularis of pharynx; o, oviduct; p, penis papilla; q, common glandular duct; r, mass of cells of female atrium; s, seminal vesicle; t, pharynx pocket; v, female genital canal (vagina); z, shell glands.



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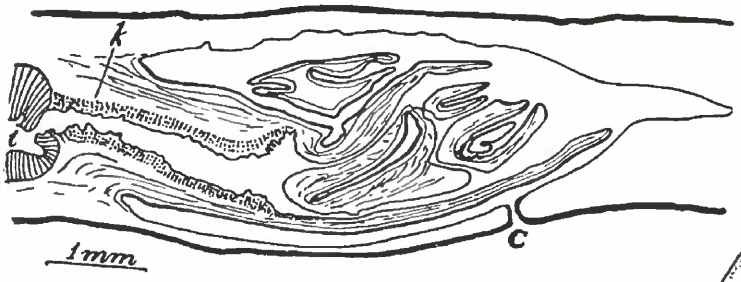

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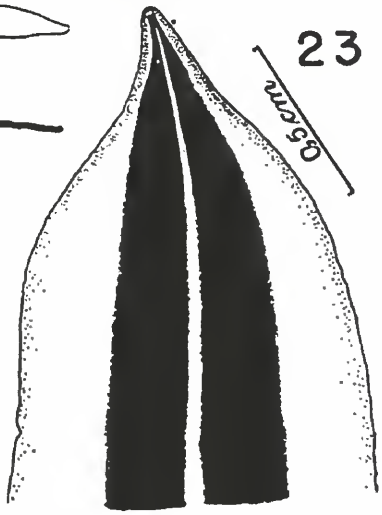

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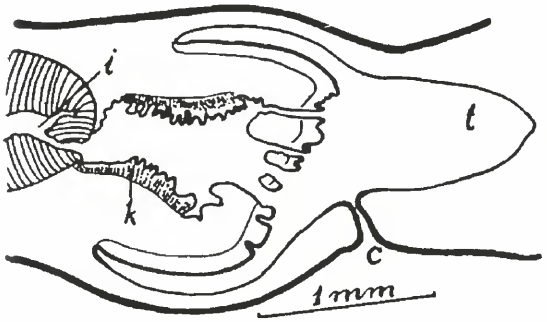

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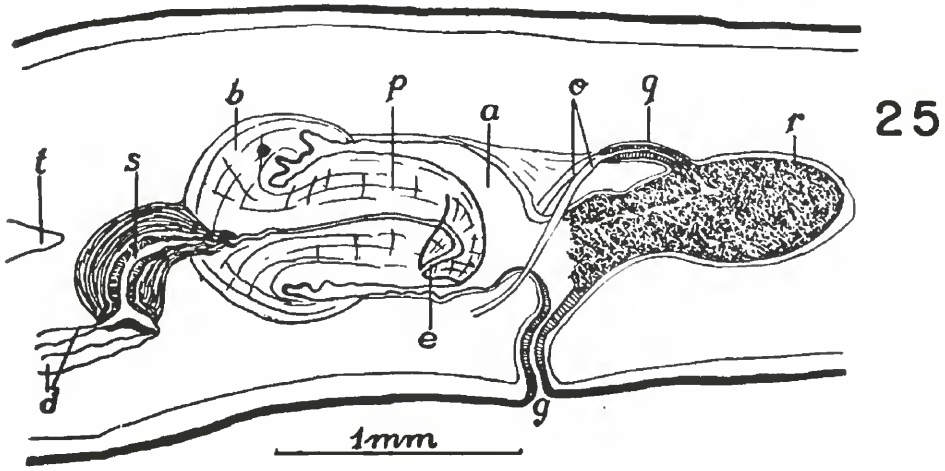

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PLATE 5

Geoplana toriba, n. sp.
(also pl. 4, Fig. 26)

Fig. 27 — Copulatory apparatus, combined sagittal sections.
Fig. 28 — Dorsal view of the preserved worm.
Fig. 29 — Distribution of the eyes at the anterior end. The tip is missing.
Fig. 30 — Distribution of the eyes farther back.

Issoca potyra, n. sp.
(also pl. 6, Figs. 35-38)

Fig. 31 — Dorsal view of the anterior end.
Fig. 33 — Distribution of the eyes at the anterior end.
Fig. 34 — Distribution of the eyes farther back.

a, male atrium; b, penis bulb; d, efferent duct; e, ejaculatory duct; f, female atrium; g, gonopore; o, oviduct; p, penis papilla; q, common glandular duct; s, seminal vesicle; y, eosinophilous glands; z, shell glands.

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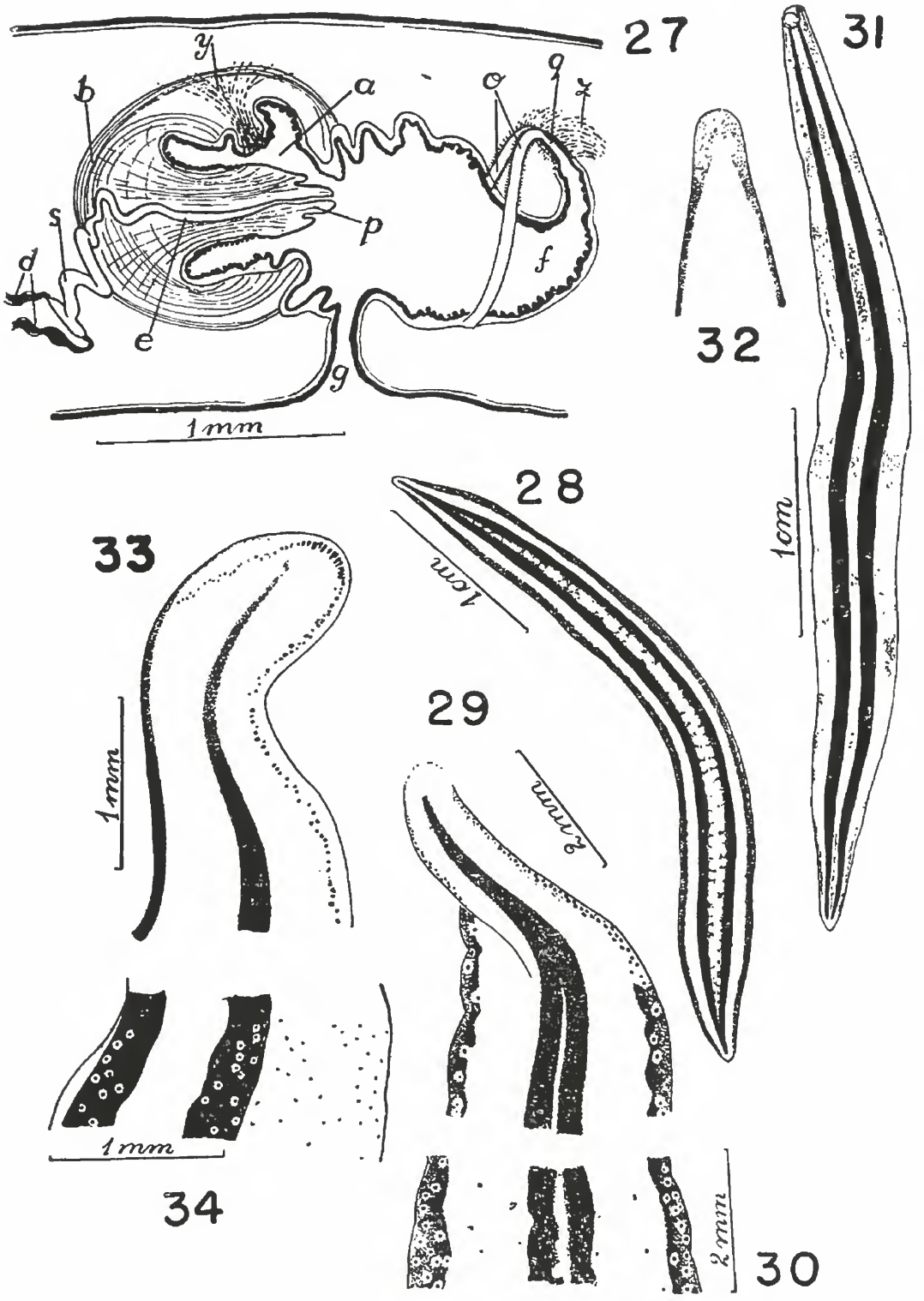


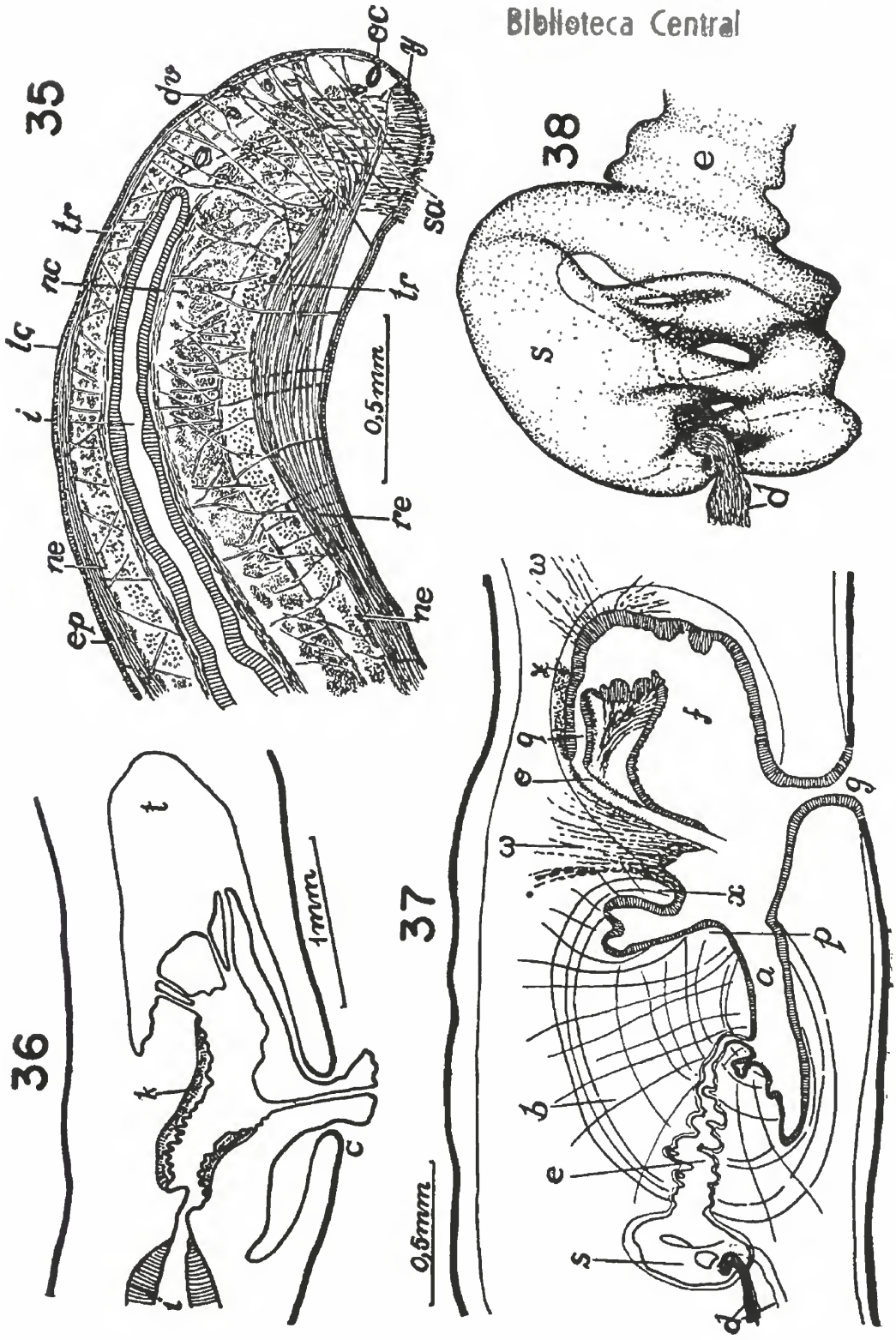
PLATE 6

Issoca potyra, n. sp.

(also pl. 5, Figs. 31-34)

Fig. 35 — Median section of the glandulo-muscular organ.
Fig. 36 — Pharynx, median section.
Fig. 37 — Copulatory apparatus, combined sagittal sections.
Fig. 38 — Detail of seminal vesicle, showing the interconnections seen from the left side.

a, male atrium; b, penis bulb; c, mouth; d, efferent duct; dv, dorso-ventral muscles; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, muscularis of pharynx; lc, subepidermal longitudinal muscles; nc, nerve plate; ne, submuscular (cutaneous) nerve plexus; o, oviduct; oc, eye; p, penis papilla; q, common glandular duct; re, retractor of glandulo-muscular organ formed by ventral subepidermal longitudinal muscles; s, seminal vesicle; sa, glandular (adhesive) surface of glandulo-muscular organ; t, pharynx pocket; tr, transverse muscles; w, cyanophilous glands; x, dorsal muscular fold separating male from female atrium; y, eosinophilous glands; z, shell glands.



ON THE ACTION OF IONS ON THE UTERINE MECHANICS

Chaim N. Grinkraut and *Paulo Sawaya*
(Department of General and Animal Physiology — Univ. S. Paulo)

1. *Introduction*

The still persisting controversy about the influence exerted by inorganic ions upon the uterine mechanics justifies a new experimental approach to the problem. Many of the results up to now obtained, which have been interpreted as probably due to the actions of Na^+, K^+, Ca^{++} and Mg^{++} ions, can be better understood only when we take into consideration other factors which can also act upon the uterus. Among those factors it must be first considered the variation of the ovarian hormones during the sexual activity that induces metabolic changes and can be responsible for the different grades of activity in the various stages of the oestral cycle and pregnancy. Secondly, it is obvious that any study of the activity of the uterine muscle depends also on the consideration of the concentration of the constituents of the bathing or perfusing fluids used in isolated experiments. Thirdly, it must be emphasized that these two just mentioned factors may have mutual influence, that is the variation of the ionic composition of the uterine muscle being correlated with the variation of the hormones (ROSSENBECK, apud REYNOLDS 1949, p. 439). Finally, in the case of action of ions, it is necessary, when a certain ion is subtracted, to consider the variation of the osmotic pressure (*o. p.*). The *o. p.* must be appropriately compensated, in order to obtain results that may really represent the effect of the removal of the ion.

The submaximal isotonic responses of the isolated uterine muscles of guinea pigs to the pituitrin extract was studied by VAN DYKE and HASTINGS (1928). They altered the ionic medium and compensated the difference of the *o. p.* by varying the NaCl content of the perfusing solution. They assumed that the small changes of NaCl would not affect the response of the guinea pig

uterus. They did not mention however the phase of the cycle of the uterus used, only saying that the work was performed with inactive muscles. This inactivity may be attributed, as we shall see later, either to the phase of complete restness during anestruous or diestruous observed in the guinea pig, or to the influence of the Mg^{++} ion, which concentration in the solution prepared by those authors is relatively high in comparison with other proposed physiological fluids.

GOMES DA COSTA (1948) discusses the question of *o.p.*, criticizing SIMONART's ideas (1926) who is inclined to assume that all the contractions (initial, after bathing and final) observed in the muscle *in vitro* are due to this factor. He sustains that these contractions should not be due exclusively to *o.p.* Other factors, such as pH, temperature, oxigenation and action of $Ca^{++} + K^{+}$ ions, should also be considered. The same has been quoted by NOVIS (1953, p. 16) who used Jalon's fluid for studying the mechanics of rat's uterus.

In GOMES DA COSTA experiments, the *o.p.* is kept constant by varying the NaCl content. When K^{+} or Na^{+} ions were removed, the Na^{+} was increased to counterbalance the absence of those ions. Inversely when the amount of Na^{+} or K^{+} was doubled, the content of the solution in Na^{+} was decreased.

It is also mentioned that DALE (1913, ap. VAN DYKE and HASTINGS, l. c.) demonstrated that small variations in *o.p.* in the bathing solution alters the responses of the uterus.

As we can see there are still doubts on the mechanism of the uterine contractions. By a method that we think better appropriated for studying the present problem we have attempted to contribute to a bettesr understanding of the mechanism referred to.

2. *Material and Methods*

Virgin female rats (Wistar) were used, weighing 180 g (± 20 g) supplied by the Instituto Butantan, Torres Laboratory and Faculty of Medicine of the University of São Paulo. A Palmer apparatus for perfusion of isolated organs was employed for graphical records at 37° C (± 1° C); the pH of all solutions was adjusted to 8 with Sodium Bicarbonate. Before each experiment, the

oestral cycle phase was checked by simple vaginal smear, stained by an aqueous 0,5% methylene blue solution. The animals were killed by a blow in the occipital region followed by isolation of the uterine horns which were immerged in 40 ml of a phsiological solution.

The procedure was the following: several experiments were performed in order to detect the influence of such ions on the uterine fibers. Care was taken to maintain the *o.p.* at the right level, because preliminary experiments have demonstrated that this factor is an essential one in the uterine contraction. After several attempts, experiments have been done as follows: a) a modified Tyrode solution (NaCl-9,00 g/1; KCl-0,42 g/1; $CaCl_2$ 0.06 g/1; $NaHCO_3$ — 0.5 g/1) in which the uterus contracts well, was taken as a standard solution. Whenever it was desired to decrease in the solution the rate of a certain ion, the variation in *o.p.* was compensated by adding glucose.

For each ion 4 solutions were prepared, each with the ion under study in different concentrations, but all of them, with the original *o.p.* and pH. In solution 1 the ion was absent; in solutions, 2, 3 and 4 its concentration was respectively 1/4; 1/2 and 3/4 of the original concentration. With this procedure it was possible to obtain records and duplicates of different stages in the different solutions used and to study the action of a certain ion on the rat's uterine muscle.

3. *Results*

We first tried to recompose Locke's and Tyrode's fluids. Only in one experiment, with Locke, we got a nearly complete return to normality when all components of the fluid were successively added (Gr. 1). Neverthless in experiments with Tyrode, or even with Locke, the muscle did not return to the original state after being bathed in a solution with only one or two of the ions.

A) *Influence of* Na^+

In every stage of the oestral cycle the complete absence of Na^+ provokes on the muscle a tendency to tetany followed by a rising of the basic line and a resting in contracture (Gr. 2). This resting is reversible, that is, by changing from the solution without Na^+ to another with 1/4 of the normal concentration of that ion,

the organs recover their normal contractions. It was further observed that as the ratio Na/K approaches the normal, the amplitude of the contractions increases and even becomes more regularly than at the beginning. This relative regularity of the contractions can be atributed to the glucose used to obtain the compensation. The effects due to increasing the Na^+ content above the normal concentration, was not considered because in those cases it is hard to separate the osmotic effects from those produced by the ion.

B) *Influence of* K^+

In all experiments on complete absence of K^+ the uterine contractions are similar to those observed in the case of Na^+, with a tendence to rest in contracture. The frequency is greater than the one observed in the case of Na^+, and the amplitude becomes lower and lower, until no contractions are finally observed (Gr. 3). This blockage of the uterine activity is also reversible, for a return of the contractions is observed when the solution without K^+ is replaced by one with only 1/4 K^+, and so on. The amplitude of the contractions increases as the rate of K^+ in the solution becomes higher and higher until the initial concentration is reached.

C) *Influence of* Ca^{++}

In every case the absence of Ca^{++} caused relaxation of the uterine muscle with a previous stage, in which the frequency increases and the amplitude decreases until complete stopping. This rest is also reversible when the solution without Ca^{++} is immediately substituted by another with Ca^{++} (Gr. 4). Prolonged immersion in a solution without Ca^{++} however, alters the muscle so much that it no longer returns to the normality when it is replaced in solutions tending to the original concentration of the ion. It is still possible to verify, by comparing the record nr. 4 with that related to the experiments with the ion K^+ (Gr. 3), that there is, in fact, an antagonism between those two ions. Actually, the absence of K^+ and the presence of Ca^{++} induced a blockage contracture; inversely the absence of Ca^{++} and the presence of K^+ has caused a relaxation.

The influence of Ca^{++} in different concentrations in the several normal physiological fluids, may be evidenced when we replace

a certain solution by another. The organ presents greater concentrations in the Locke's fluid in which Ca^{++} is 4 times more concentrated (0.24 g/1) than in the modified Tyrode (0.06 g/1).

As to the greater amplitude of the contractions in Locke's fluid than in Tyrode's this might be due not only to the different concentrations of Ca^{++} in both solutions as well to the absence of the ion Mg^{++} in Locke's fluid. The presence of Mg^{++} decreases not only the amplitude, but also the frequency of the contractions.

D) *Influence of Mg^{++}*

The absence of Mg^{++} does not alter appreciably the mechanics of the isolated uterus of the rat, when other ions are present in their convenient ratios. The experiments were repeated adding Mg^{++} to the standard fluid. The results are recorded in graph nr. 5 in which the preceeding phenomena can be well observed. It must be only emphasized the important fact that the presence of Mg^{++} only delays the blockage, in contracture, in the absence of Na^{+} and K^{+}, and in relaxation in the absence of Ca^{++}. These conclusions are reinforced by the fact that when the uterine muscle is bathed in a solution without Mg^{++} and Na^{+}, the mentioned stopping in contracture is verified after 65 minutes; and when the Mg^{++} is present in the solution this resting takes place after 122 minutes without modification of the main phenomena.

In the case of K^{+} we have:

Solutions without Mg^{++}	—	contracture after	54	minutes
Solution with Mg^{++}	—	" "	77	"

In the case of Ca^{++}

Solutions without Mg^{++}	—	contracture after	54	minutes
Solution with Mg^{++}	—	" "	124	"

4. *Discussion*

Excepting the works of VAN DYKE and HASTINGS and GOMES DA COSTA, previous papers on the action of ions on uterus have not taken into consideration the influence of the factor *o.p.*

Accordingly, we shall compare our results, obtained with rat, with those obtained by the authors above mentioned.

In the GOMES DA COSTA's experiments the *o.p.* is maintained constant, as already mentioned, by the variation of the NaCl content the physiological fluid. From the description of the results and the reccrds obtained by this author, the ion Na^+, in the compensation, should have the same influence as the Mg^{++} ion in ours, i. é, it only delays the blockage provoked by the absence of K^+ and the relaxation if Ca^{++} is absent from the fluid. We think that this type of compensation is an incorrect one because when we increase the rate of one of the ions and decrease the rate of another, the ionic equilibrium is broken, and also its respective ratios, as may be seen in the case of the Na^+/K^+ ratio.

The correction of the *o.p.* by glucose has given better results, having in addition the advantage of regulating the rithm and the amplitude of the contractions.

On the other hand, van DYKE and HASTINGS' work differs primarily from ours in the fact that they used guinea pig's uterus in an inactive stage, that is, when it does not contract spontaneously. Using the VAN DYKE and HASTING's fluid, (as they proposed) the rat uterus, active in Locke or Tyrode's, showed very litlte activity. It would be interesting to know the behaviour of the guinea pig uterus in Tyrode, but this was not done by those authors. The inactivity of the rat's uterus in the Van Dyke's solution could be explained by the antagonism between Ca^{++} and Mg^{++}. In fact, analysing the ratios between Na^+, K^+, Ca^{++} and Mg^{++} in the modified Tyrode used by us and the Van Dyke's fluid we have

	Tyrode	*van Dyke-Hastings*
NaCl	9.00 gr/1	6.595 gr/1
KCl	0.42 gr/1	0.462 gr/1
$CaCl_2$	0.06 gr/1	0.058 gr/1
$MgCl_2$	0.05 gr/1	0.095 gr/1
Na/K ratio =	21,4	Na/K = 14,2
Mg/Ca " =	0,083	Mg/Ca = 1,637

One can see a marked difference in the ionic relation between the two solutions in relation to the quantities of Na^+ and Mg^{++}. The quantity of Ca^{++} in both solutions is almost the same, but that of Mg^{++} is larger in the Van Dyke-Hasting's solution. During our experiments we observed that Mg^{++} in fact is a modifier of the action of Ca^{++} upon the uterine muscle (Gr. 6), lowering its action. Now, the same quantity of Ca^{++} existing in both solution opposing its action to different quantities of Mg^{++} is likely to provoke different responses on the muscle. Inversely, a greater quantity of Mg^{++} acting on a same quantity of Ca^{++} can bring up the muscle to an inactivity, what, in fact, was verified. As Locke's fluid does not contain Mg^{++}, it is out of consideration here. The greater amplitude of the contraction of the uterus in Locke's solution may be attributed to the absence of the Mg^{++} ion. In this case the great quantity of Ca^{++} does not find any opposition to its action.

In relation to the action of the Na^+ ion on the uterine mechanics, only VAN DYKE and HASTINGS paper says something about: the increase of the Na^+ ions induces a greater excitability on the guinea pig's uterus to the pituitary extracts, and when the concentration of all ions is reduced to a half, with the exception of Na^+, the response is small but greater than when Na^+ is also reduced to a half. The results obtained in our experiments have been already analysed during the description of the action of this ion.

When the *o.p.* is not counterbalanced, the complete absence of Na^+ or its presence in a concentration equal to the half of the original quantity, provokes a relaxation of the uterine muscle, and this is just the contrary of what is observed when the *o.p.* of the solution is mantained at the right level (Gr. 7). The ratio Na^+/K^+ is equal 13.66 in the experiments in which the concentration of Na^+ is half of the original, and without compensation, and in the ones in which there was compensation. Therefore the results differ when the uterine muscle of the virgin rat is placed in different ionic and osmotic conditions.

When the ratio Na^+/K^+ is varied and *o.p.* is properly compensated, the results are different from the ones obtained in the

experiments in which the referred ratio is the same, while *o.p.* is not counterbalanced. Our results are similar to those obtained by GOMES DA COSTA in the case of the complete absence of K^+ in Tyrode's solution, although the composition of Tyrode solution then used is not indicated.

It must be emphasized that in those experiments, GOMES DA COSTA did not really compensate the ion withdrawal, because when 0.012% of NaCl was added the expected rest came later. When the rate of K^+ was twofold without compensation was employed the uterus rested after 4.5 hours, and with compensation only after 7.5 hours.

When a twofold, or fourfold of ion concentration is used as it was done by GOMES DA COSTA, the balanced ratio of the ions is strongly altered, mainly the ratios Na^+/K^+ and K^+/Ca^{++}; on the other hand, it seems not reasonable that the compensation should be always made in the same way,, that is, the withdrawal of one ion is compensated by adding 0.012% of NaCl while his twofolding ou fourfolding is compensated with a decreasing of 0.012% NaCl.

In the fourfolding of the ion K^+, a rest is observed after 2 to 2.5 hours. In the experiments without compensation with overpassing normal concentration of the ion, the results were the following:

a) concentrations up to 1.4g/1 (nearly 3.5 times the normal) stimulate the uterine contractions; b) 1.5g/1 induces a blockage and c) 1.7g/1 provokes a sudden and complete blockage (1.7 = fourfold). The time for the complete blockage was never superior to 30 minutes, as can be seen in record nr. 8.

Apud CLARK, KNAUS and PARKES (1926), twofolding ou threefolding the amount of KCl of Locke's fluid (which is the same as in the Tyrode's) has an effect almost of estimulation of the rat's uterus identical with that produced by the pituitary extracts. The excess of KCl causes an increasing in the frequency of the contractions, while its withdrawn provokes also as an early effect, that is, an increasing of the frequency of the contractions.

In the first case there is a facilitation in the conduction, resulting in contractions of higher amplitude; in the second, there occurs a decrease in the rate of conduction, the contractions being of lower amplitude.

BLAIR-BELL and co-workers verified that the increasing or decreasing of theK^+ contents within reasonable limits, do not produce remarkable alterations of the uterine movements. *In vivo* injections 2 ml of a solution 5% KCl have produced discordant results even in the same animal, for they observed either stimulation or inibition of the contraction.

VAN DYKE and HASTINGS using the pituitary extracts as stimulator, verified that the reduction or the complete withdrawn of the K^+ ions causes a decreased response to the pituitrine (inactive uterus). They observed that generally an increasing in the K^+ concentration is sufficient to alter the response of the uterus to pituitrin, starting a sequency of rithmic contractions.

Ca^{++} ions are considered specific for the uterus, as already shown. The isolated uterine muscle is more susceptible to Ca^{++} than to the other ions. The addition of Ca^{++} to the bath induces activity in an inactive uterus. Referring to the absence of Ca^{++}, all authors agree that it brings the uterine muscle into relaxation. Above the normal rate Ca^{++} provokes a resting in contracture. GOMES DA COSTA doubling the rate of Ca^{++} (without compensation) got a stop in contracture, which was longer when he used compensation. The same consideration about this ion can be made in the case of the ion K^+. VAN DYKE and HASTINGS also did not detect any response to pituitrin, when Ca^{++} is missing in the solution. The responses decrease when the Ca^{++} content decreases in the fluid. Inversely, increasing the concentration of the ion an increasing of the contractions is also observed.

BLAIR-BELL and co-workers suggested that the quantity of Ca^{++} in the blood below the normal is probably a factor that causes a simple primary inertia.

In vitro it was demonstrated that varying the rate of Ca^{++} to 25%, 33%, 50%, 66% an 90% less than in the normal Locke's fluid, the contractions becomes lower and lower.

The results here obtained with the Mg^{++} ion suggests that that ion is a modifier of the Ca^{++} ion action. It also antagonizes, partially, the Na^+ and the K^+ action, because it is capable of brin-

ging about a relaxation of a muscle in contracture or, at least, to delay the blockage.

REYNOLDS in living unanesthetized rabbits, verified that the Mg^{++} ion inhibits the sustained contraction induced by Ca^{++} and prevents any further answers.

4. *Summary*

1) The action of Na^{+}, K^{+}, Ca^{++} and Mg^{++} on the mechanics of the albino rat (Wistar) uterus was studied. Whenever a certain ion concentration was decreased, the osmotic effect was compensated with glucose so that throughout the experiments a same osmotic pressure of the perfusing fluid was maintained.

2) Of each of the ions studied, four solutions were prepared: normal, one quartel, half and three quarters of the original concentration. Care was also taken to maintain the same pH in all cases. The following results were obtained, when a modified Tyrode (Mg free) was taken as a starting point, and during the various phases of the oestrous cycle.

a) Absence of Na^{+} induces a blockage in contracture, reversible as the concentration of the ion gradually increases again. With 1/4 of its original concentration spontaneous contractions reappear. (Gr. 2).

b) Absence of K^{+} causes a sustained contraction similar to that obtained in the absence of Na^{+}, but whereas in the case of Na^{+} the height of the contractions prior to contracture is not altered, in the case of K^{+} a tetanic type of contracture occurs. As K^{+} increases again contractions reappear. With 1/4 they return to the basal line, but curiously with half and with three quarters there occured a decrease in amplitude and frequency. The complete Tyrode finally restored the normal rhythm.

c) Absence of Ca^{++} induces a complete relaxation of the uterus and prolonged immersion in calcium free Tyrode makes impossible the return to normality. This one can be obtained if immediately after exposing the uterus to a soluction free form Ca^{++} increasing concentrations of the ion are used.

d) Ausência de Mg^{++} não induz mudanças perceptíveis no ritmo do útero pois não foram observadas diferenças quando se usou Tyrode completo. Entretanto, em presença de Mg^{++} as contraturas obtidas com ausência de Na^{+} ou K^{+} e o relaxamento induzido pela ausência de Ca^{++}, se tornam grandemente retardadas.

5. *Resumo*

1) Foram estudadas as ações dos ions Na^{+}, K^{+}, Ca^{++} e Mg^{++} sôbre a mecânica uterina de ratos albinos (Wistar). Sempre que a concentração de um certo ion foi diminuída, o efeito osmótico foi compensado com glicose, a fim de que fôsse mantida a mesma pressão osmótica (o. p.) em tôdas as experiências.

2) De cada um dos ions estudados foram preparadas 4 soluções: normal, 1/4, 1/2 e 1/3 da concentração original. Tomou-se cuidado para que o mesmo pH fôsse mantido em todos os casos. Usou-se como solução fisiológica um Tyrode modificado (sem Mg). Os seguintes resultados foram obtidos durante as várias fases do ciclo estral.

a) Ausência de Na^{+} provoca um bloqueio em contratura que é reversível à medida que a concentração do ion aumenta gradualmente. Com 1/4 da sua concentração original as contrações espontâneas reaparecem.

b) Ausência de K^{+} causa uma contratura sustentada semelhante àquela obtida na ausência de Na^{+}, mas enquanto que no caso do Na^{+} a altura das contrações anteriores à contratura não é alterada, no caso de K^{+} ocorre um tipo de contratura tetânica. À medida que a concentração de K^{+} aumente novamente, as contrações reaparecem. Com 1/4 da concentração normal elas voltam à linha de base. Curiosamente, com 1/2 e 3/4 ocorreu uma diminuição da amplitude e freqüência. O Tyrode completo restabeleceu o ritmo normal.

c) Ausência de Ca^{++} induz ao completo relaxamento do útero e a permanência prolongada num Tyrode sem Ca torna impossível a volta à normalidade, a qual pode ser obtida se imediatamente após a exposição do útero à ausência de Ca, se usam concentrações progressivamente maiores do ion.

d) Absence of Mg^{++} induces no noticeable changes in the rhythm of the uterus, for no differences were observed when complete Tyrode was used. However, in the presence of Mg^{++}, the contracture obtained in the absence of Na^{+} or K^{+} and the relaxation induced by the absence of Ca^{++} are greatly delayed.

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GRÁFICOS

GRAPH 1

S_1 = Locke

NaCl	.....	9.15 g/1
KCl	.....	0.42 g/1
$CaCl_2$	.....	0.24 g/1
$NaHCO_3$	.....	0.15 g/1
Glicose	.....	1.00 g/1

S_2 = 25 ml NaCl ..... 91.5%.

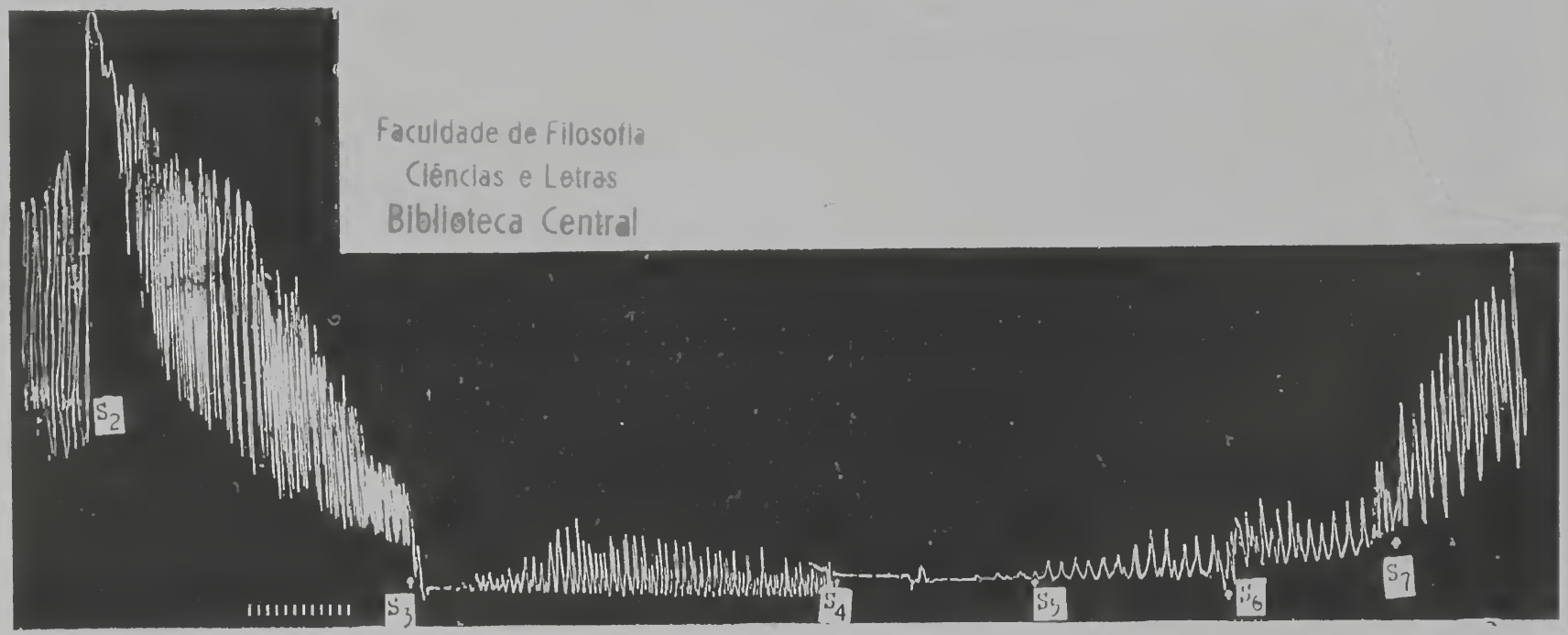
S_3 = S_2 + 2.5 ml KCl .... 4.2%.

S_4 = S_3 + 50 ml $CaCl_2$ .... 0.24%.

S_5 = S_4 + 0,25 g glucose

S_6 = S_1

S_7 = S_1

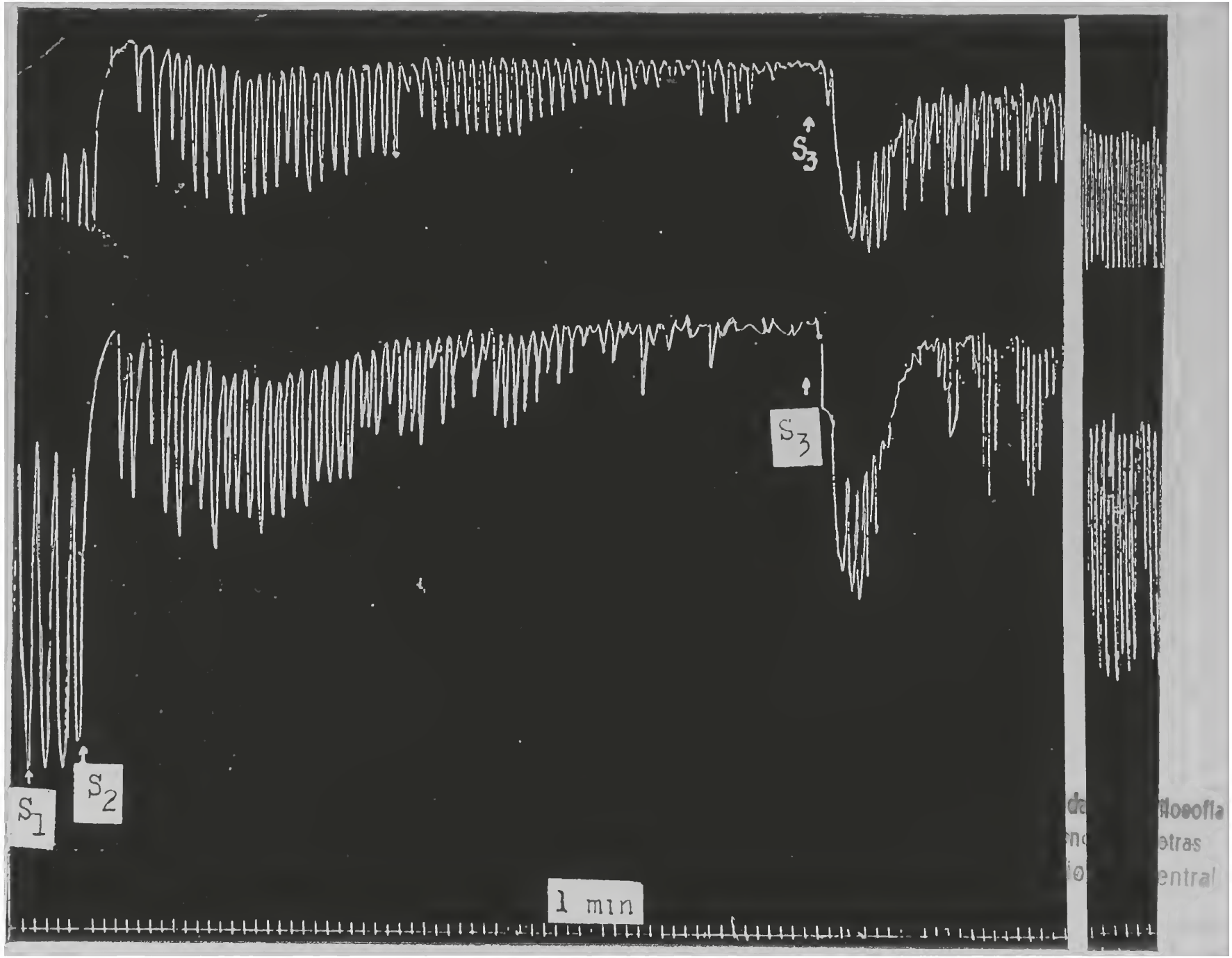


GRAPH 2

S_1 =	NaCl	9.00g/1
	KCl	0.42g/1
	$CaCl_2$	0.06g/1
	$NaHCO_3$	0.5g/1

$S_2 = S_1 - Na + 51.3g/1$ glucose

$S_3 = S_1 + 1/4\ Na + 38.4g/1$ glucose


S3
S3
S1
S2
1 min

GRAPH 3

S_1 =	NaCl	9.00g/1
	KCl	0.42g/1
	$CaCl_2$	0.06g/1
	$NaHCO_3$	0.5 g/1

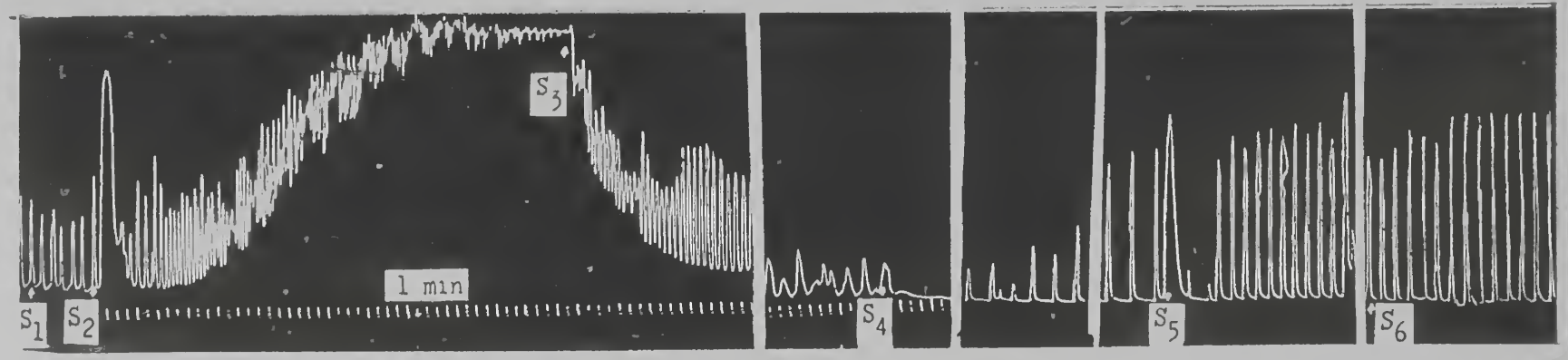
$S_2 = S_1 - K + 0.18g/1$ glucose

$S_3 = S_1 + 1/4\ K + 0.135g/1$ glucose

$S_4 = S_1 + 1/2\ K + 0.09\ g/1$ glucose

$S_5 = S_1 + 3/4\ K + 0.045g/1$ glucose

$S_6 = S_1$



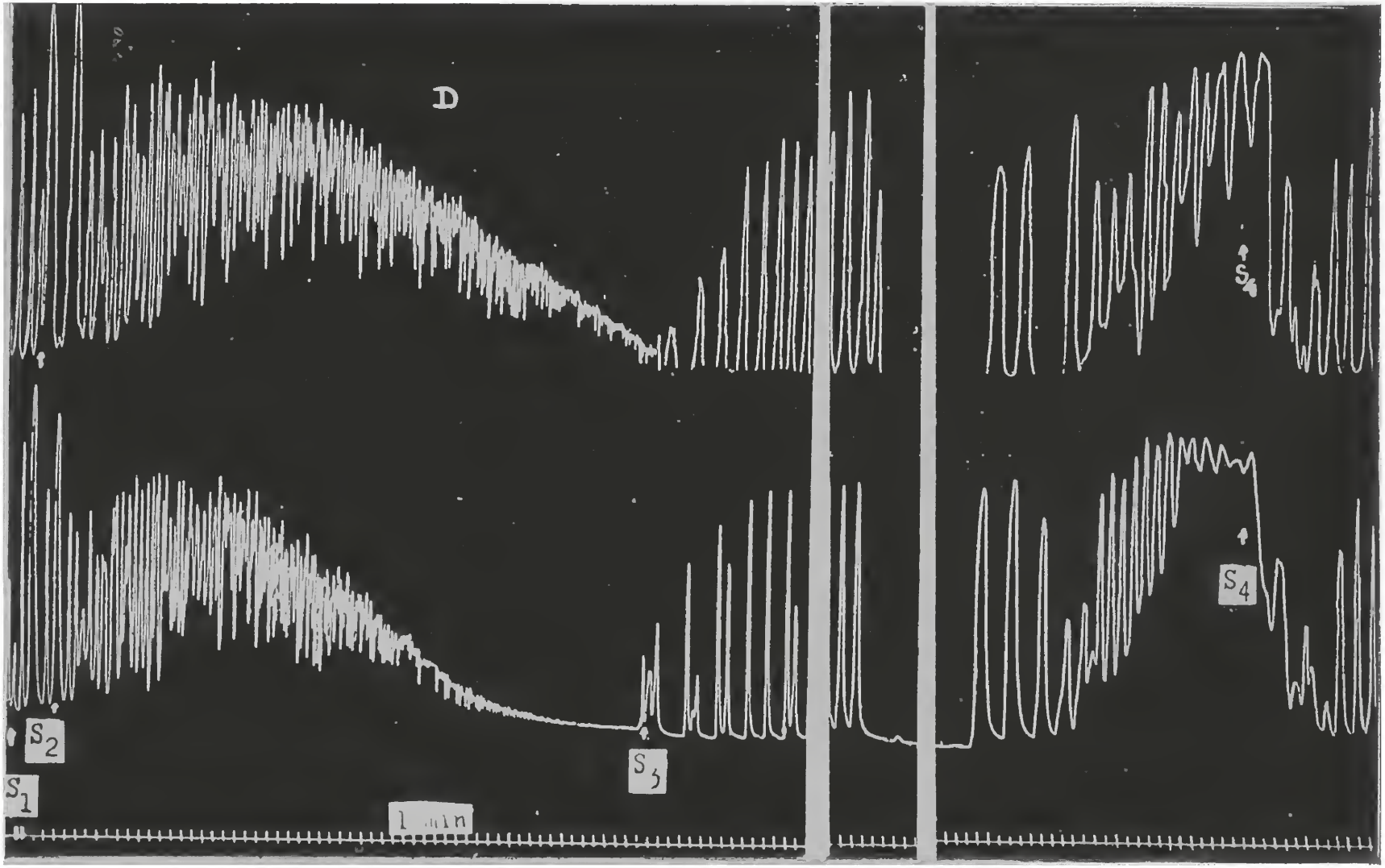
GRAPH 4

S_1 = NaCl	.....	9.00g/1
KCl	.....	0.42g/1
$CaCl_2$	.....	0.06g/1
$NaHCO_3$	.....	0.5 g/1

S_2 = S_1 − Ca + 0.198g/1 glucose

S_3 = S_1 + 3/4 Ca + 0.049g/1 glucose

S_4 = S_1

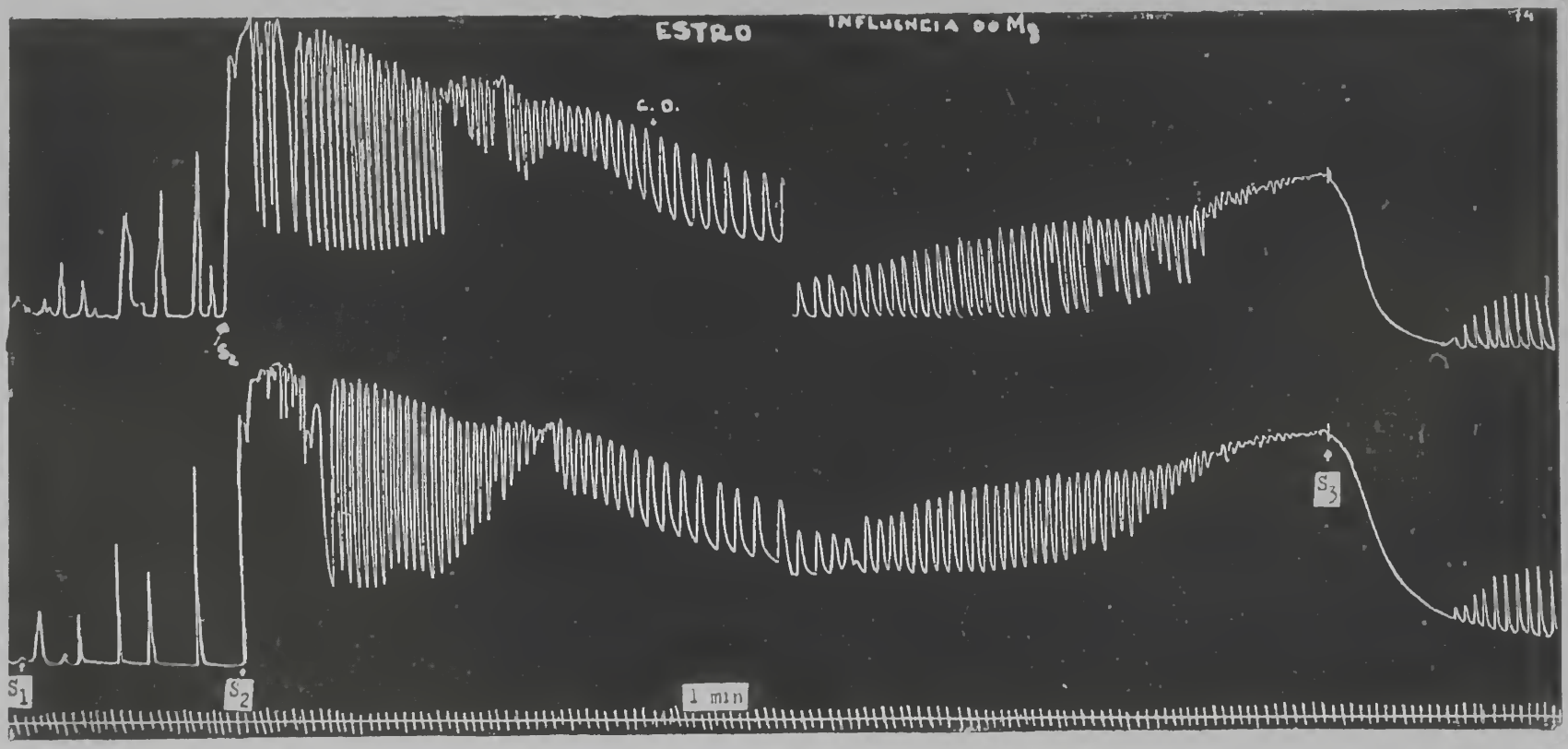


GRAPH 5

S_1 = NaCl	.....	9.00 g/1
KCl	.....	0.42 g/1
$CaCl_2$	.....	0.06 g/1
$MgCl_2$	.....	0.005g/1
$NaHCO_3$	.....	0.5 g/1

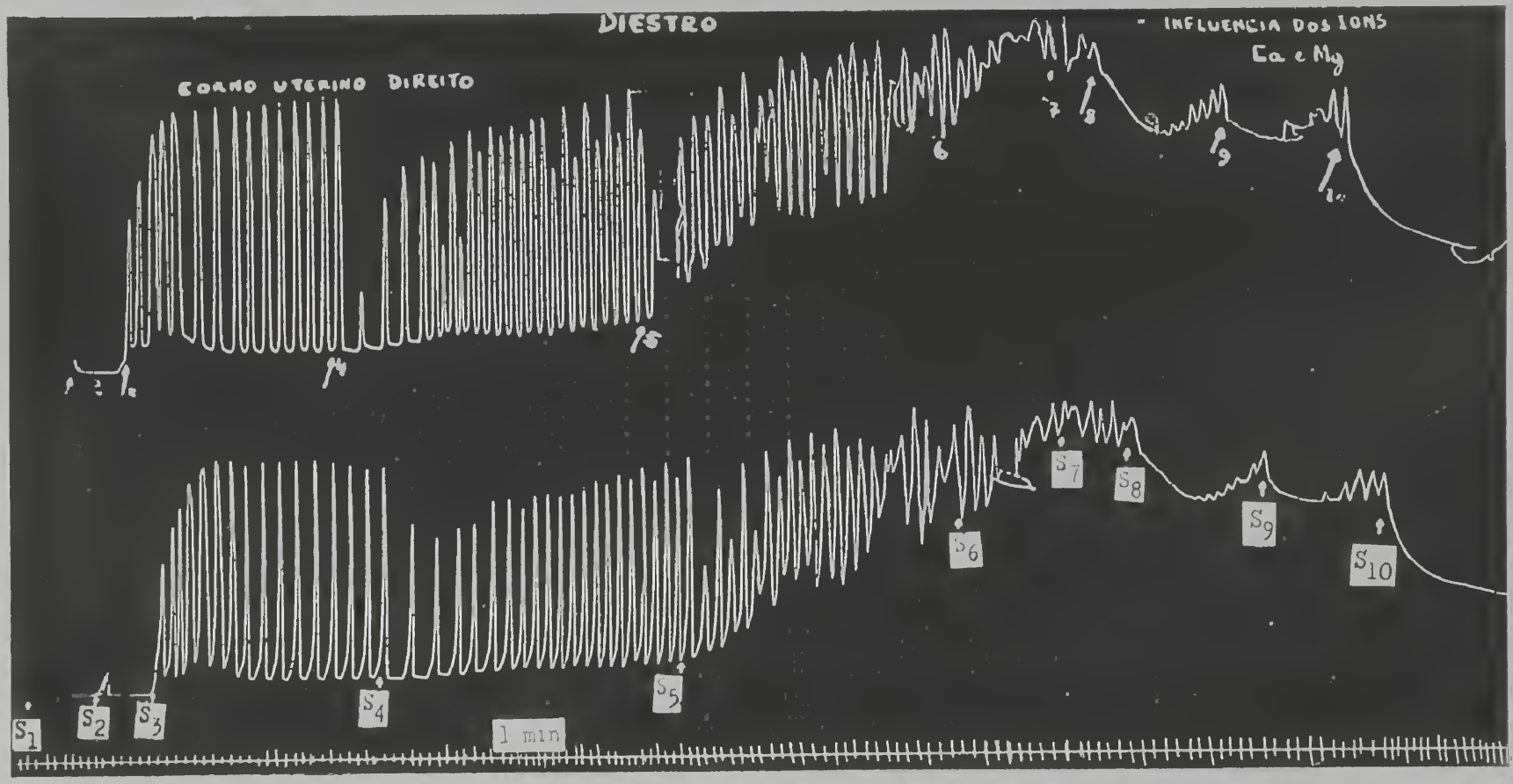
$S_2 = S_1 - Na + 51.3g/1$ glucose

$S_3 = S_1 + 1/4\ Na + 38.4g/1$ glucose



GRAPH 6

$S_1 = S_2 =$	NaCl	9.00g/1
	KCl	0.42g/1
	$CaCl_2$	0.06g/1
	$NaHCO_3$	0.5 g/1
$S_3 = S_2 +$	1 ml $CaCl_2$ 4%	
$S_4 = S_3 +$	0.5 ml $CaCl_2$ 4%	
$S_5 = S_4 +$	0.5 ml $CaCl_2$ 4%	
$S_6 = S_5 +$	0.5 ml $CaCl_2$ 4%	
$S_7 = S_6 +$	2 ml $MgCl_2$ 2%	
$S_8 = S_7 +$	5 ml $MgCl_2$ 2%	
$S_9 = S_8 +$	5 ml $MgCl_2$ 2%	
$S_{10} = S_9 +$	10 ml $MgCl_2$ 2%	

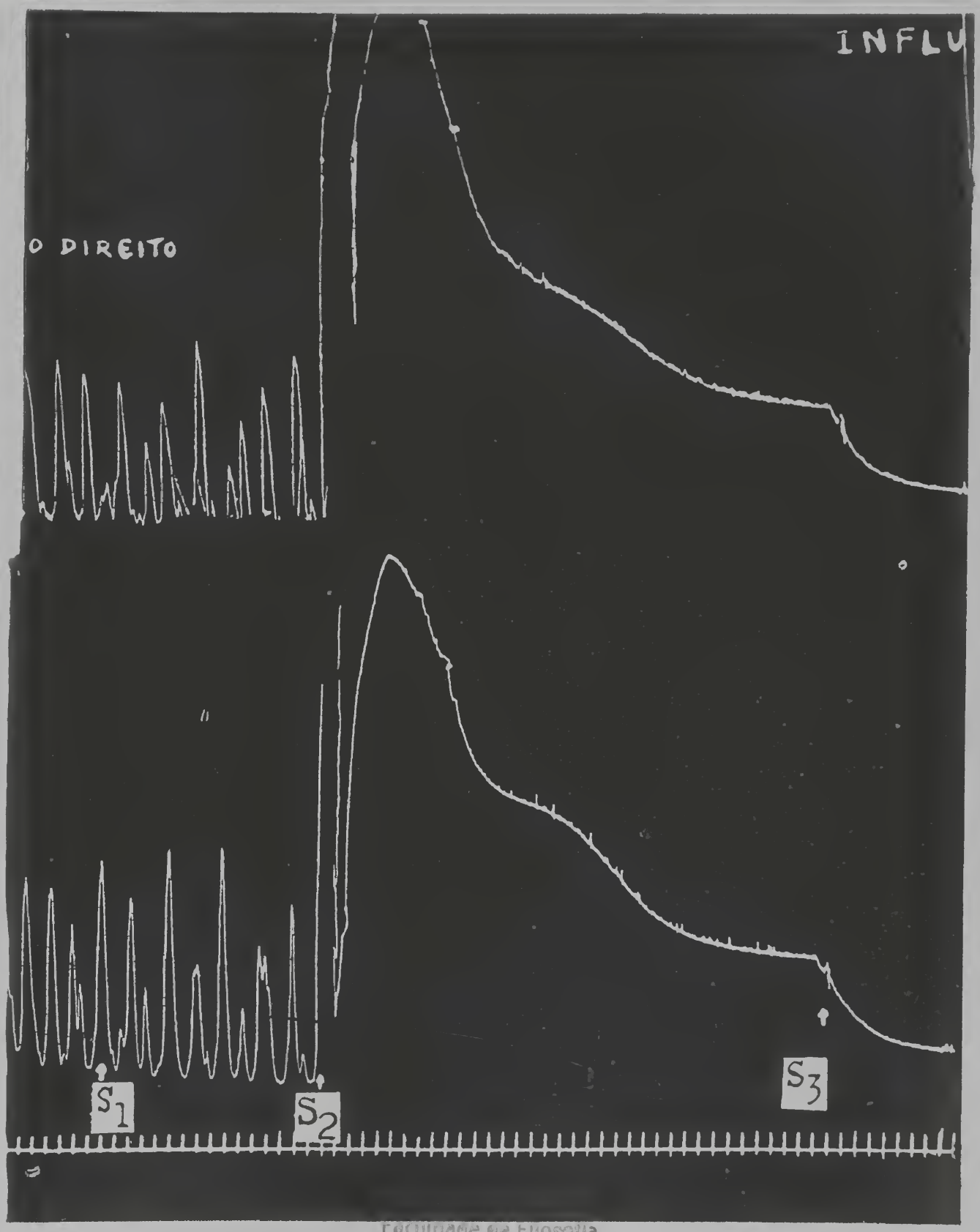


GRAPH 7

S_1 = NaCl	.....	9.00 g/1
KCl	.....	0.42 g/1
$CaCl_2$	.....	0.06 g/1
$MgCl_2$	.....	0.005g/1
$NaHCO_3$	.....	0.5 g/1

$S_2 = S_1 - NaCl$

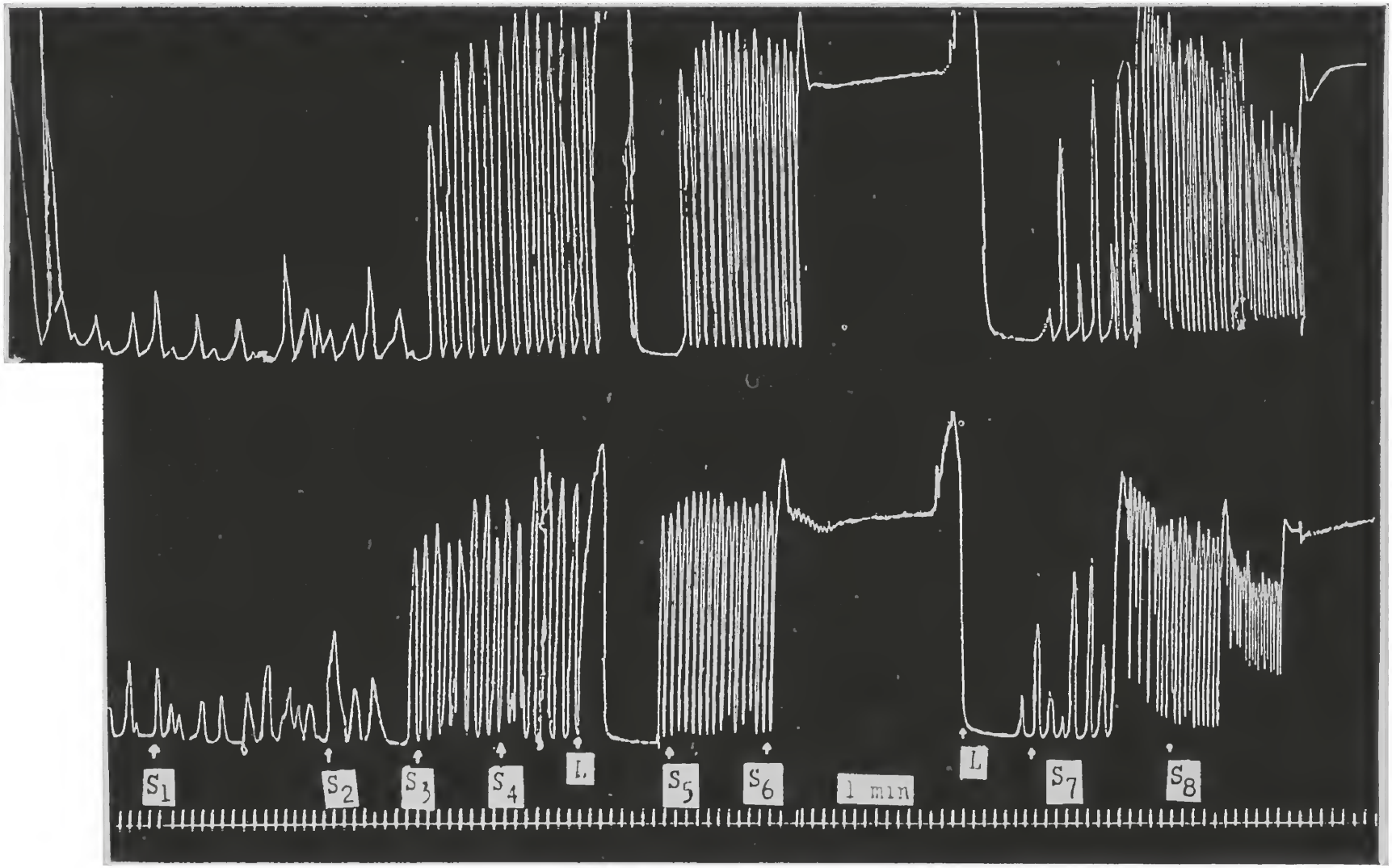
$S_3 = S_2 + 1$ ml NaCl 9.g/1



GRAPH 8

S_1 = NaCl 9.00 g/1
KCl 0.42 g/1
$CaCl_2$ 0.06 g/1
$MgCl_2$ 0.005g/1
$NaHCO_3$ 0.5 g/1

S_2 = S_1 + 1 ml $CaCl_2$ 0.24%
S_3 = S_1 0.5 ml KCl 4.2%
S_4 = S_1 + 0.5 ml KCl 4.2%
L = washing with S_1
S_5 = S_1 + 1 ml KCl 4.2%
S_6 = S_5 + 0.5 ml KCl 4.2%
L = washing with S_1
S_7 = S_1 + 1 ml KCl 4.2%
S_8 = S_1 + 0.5 ml KCl 4.2%



THE RESPIRATORY METABOLISM OF TROPICAL EARTHWORMS

II. Studies on the cutaneous respiration.

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INTRODUCTION

Respiratory exchange in the Oligochetes takes place generally through the body wall (STEPHENSON 1930, p. 181). This respiration may consist simply in a direct exchange through the parietes with an entire absence of parietal blood vessels (*Chaetogaster* and *Aeolosoma*) or the exchange may be facilitated by the presence of blood vessels. These may occur in a copious network on the inner face of the body wall, the vessels being applied to or branching on the inner face of the body wall without penetrating the parietes (many Limicolae) or parietal vessels may penetrate the substance of the body wall, sometimes sending capillaries loops into the epidermis. The last situation is found, as a rule, in all Oligochetes where the body wall is of any considerable thickness, but can also occur in thinwalled small Tubificidae.

The penetration of the epidermis by capillaries in Oligochotes has been known for a long time. CLAPAREDE (1869) in *Lumbricus* described that in the clitellar region the terminal branches of the vascular system push their way in among the cells, forming loops, which, however, stop short some little way below the surface. As to the vascularity of the rest of the epidermis, BEDDARD (1895, p. 75) claims to be the first to have pointed out that this was the case with the Megascolecidae *Pleurochaeta Moseley* (*Megascolex coeruleus*), some species of *Perichaeta* (*mostly Pheretima*) and with the Tubificidae *Limnodrilus*. In 1887, howe-

ver, ROSA (apud STEPHENSON l. c.) described blood capillaries in the epidermis of an aquatic Glossoscolecida (*Criodrilus lacuum*). Intraepidermal capillaries in Oligochetes have since been described in the Tubificidae again by NOMURA (1913, p. 25) and MARCUS (1942, p. 180), in species of Moniligastridae of the genus *Drawida* (apud STEPHENSON l. c., p. 183), in aquatic Glossoscolecidae such as *Alma nilotica* (GRESSON 1927) and in the terrestrial Megascolecida *Dichogaster budgetti* (BEDDARD 1900, apud STEPHENSON l. c., p. 183). As to the Lumbricidae, LENHOSSEK (1895, apud STEPHENSON l. c., p. 183) described intraepidermal vessels in *Lumbricus*, at some distance behind the clitellum, whereas SZÜTS (apud STEPHENSON l. c., p. 184) found an abundant system of epidermal capillary loops in *Allolobophora dubiosa*. No reference could be found in the literature with regard to the forms which are commonly found in Brazil, such as the Glossoscolecidae *Glossoscolex* and *Pontoscolex* and the Megascolecida *Pheretima*. As to the latter, although BEDDARD (l. c., p. 75) mentioned the occurrence of intraepidermal vessels in "Perichaeta", which might include some species of *Pheretima*, not even BAHL (1936, p. 35) in his Monograph on *Pheretima posthuma* is explicit enough about the morphological features of these blood vessels.

In the course of a series of studies on the respiratory metabolism of tropical earthworms, after a previous investigation of the normal respiratory rate, of the role of haemoglobin and of the relation between body size and respiratory metabolism (MENDES AND VALENTE 1953), the question of the cutaneous respiration assisted by epidermal capillaries emerged naturally. This paper reports the results from both morphological and physiological researches on the cutaneous respiration of our common earthworms. Morphological studies were performed in *Glossoscolex sp.*, *Pontoscolex sp.* and *Pheretima hawayana*. *Pontoscolex* and *Pheretima* were used in the experiments. Details of the techniques employed will be given below.

EXPERIMENTS AND RESULTS

a. *The intraepidermal capillaries of Glossoscolex, Pontoscolex and Pheretima*. In order to find out whether or not the ear-

thworms under investigation possessed intraepidermal capillaries, the animals were fixed in Bouin, after previcus cleaning of the intestinal tract, and sectioned transversally. The sections were stained with hematoxilin Regaud. This histological prccedure was good enough to make clear the existence of intraepidermal capillaries in the worms. Therefcre, we did not make use of the sc called double method of Cajal employed by LENHOSSEK (l. c.).

Fig. 1 of the table I shows a transverse section from a region in the neighborhood of the clitellum of *Glossoscolex*, where the intraepidermal capillaries can be clearly seen in a relatively dense network. Figs. 2 and 3 of the same table show enlargements of another sections from the same region, where the shape of the epidermal loops can be better followed. The loops are double and the ascending limb divides about two thirds of the height of the epidermis in the manner of the letter Y, each branch of the Y forming a separate loop. Although it is not clear in the figures, the descending limbs of the loops unite again at the same level where they originated. These double loops were also found by LENHOSSEK (l. c.) in *Lumbricus*. However, he also found single loops which could not be detected in *Glossoscolex*.

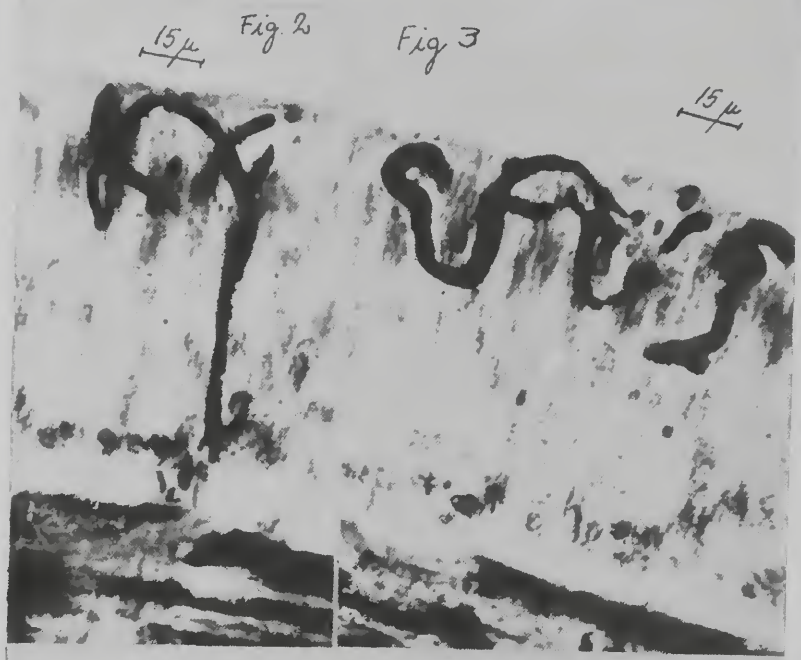
Fig. 4 of table I shows a transverse section of *Pontoscolex* also from a region in the vicinity of the clitellum. It can be seen that the epidermal loops in this earthworm are also double and resemble in shape those of *Glossoscolex*. No single loops were also found here.

Fig. 5 of table I shows, finally, a transverse section through a region near the clitellum of *Pheretima*. The loops here are single and can be followed clearly from the basis of the epidermis. No double loops could be found.

In connection with the shape of the epidermal capillaries, it is interesting to note that in *Pheretima*, a member of the family Megascolecidae, the loops are single; in *Glossoscolex* and *Pontoscolex*, members of the fam. Glossocolecidae, the loops are double; and, finally, in *Lumbricus*, of the family Lumbricidae, the loops are single and double, according to LENHOSSEK.

b. *The action of external use of adrenalin and acetylcholine on the respiratory rate of Pontoscolex and Pheretima.* The fact that

Fig. 1
Glossoscolex sp.
(near clitellum)



Figs. 2 & 3
Glossoscolex sp.
(near clitellum)
(Enlargements)

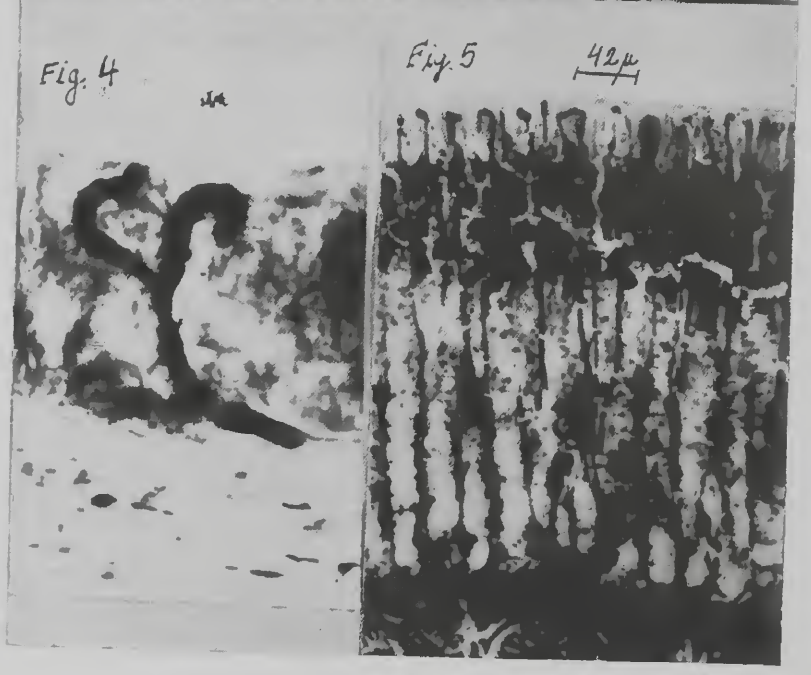


Fig. 4
Pontoscolex sp.
(near clitellum)

Fig. 5
Pheretima hawayana
(near clitellum)

TABLE I. Intraepidermal capillaries in Brazilian earthworms.

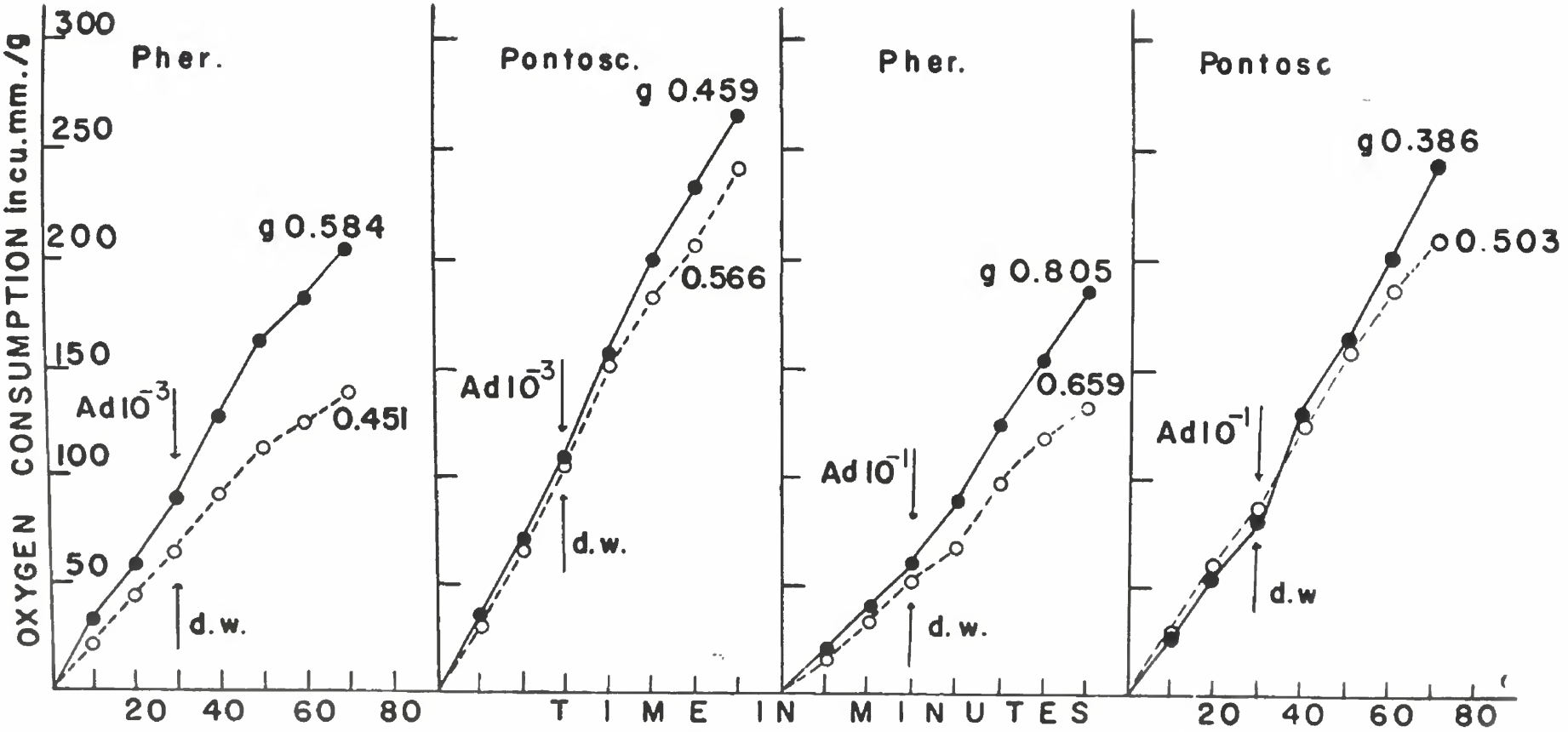


Fig. 6: The action of adrenal'n on the intraepidermal bloodvessels of of the earthworms *Pheretima* and *Pontoscolex*, as judged from the modification of the respiratory rate. The arrows indicate when the drug was tipped on the animals.

the Oligochetes studied exhibited intraepidermal capillaries suggested that there might be a peripheral control of respiration through variations in the caliber of these blood vessels and invited us to submit the animals to the external action of drugs having a known effect on capillaries.

The technique used to measure the respiration of the worms was in general similar to that of the previous work of the series (MENDES & VALENTE l. c.). The only modification was that a certain amount of the drug solution was added to the side bulbs of the Warburg vessels and care was taken to keep the animals from entering them by obstructing their entrance with a piece of filter paper. After placing the animals in the chamber, 12% KOH was added to the center well and 0.5 cc. of distiled water were pipetted into the side-bulb of half of the vessels (blanks) and 0.5 cc. of drug solution added to the bulb of the other half (experimental). The temperature of the bath was $25 \pm 0.5°C$, the vessels were gently and slowly shaken and the whole apparatus covered with a thick black cloth. After measuring the respiration during 15 minutes with 5 minute interval readings, the contents of the side-bulbs were tipped upon the worms and the eventual changes in the oxygen uptake were followed during the course of one hour.

Adrenalin (Parke and Davis Co.) and Acetylcholine (Roche Products Co.) were used as the substances to act upon the capillaries. Acetylcholine solutions were made up from the solid substance. Adrenaline solutions from the 10^{-3} solution contained in the ampules. When 10^{-1} Ad was prepared, solid substance also was employed. After the experiments the animals were dried and weighed. As a rule they endured the drug treatment and even when concentrations as strong as 10^{-1} were used they were at the end of the experiment apparently quite well.

Adrenaline was tested in the following concentrations: 10^{-5}, 10^{-3} and 10^{-1}. With 10^{-5} no changes in the respiratory rate were observed. With 10^{-3} (Fig. 6 graphs a and b) it is difficult to speak of any marked change in the oxygen uptake. With 10^{-1} very definitely, however, an increase of the respiratory rate took place both in *Pheretima and Pontoscolex* (Fig. 6, graphs c and d).

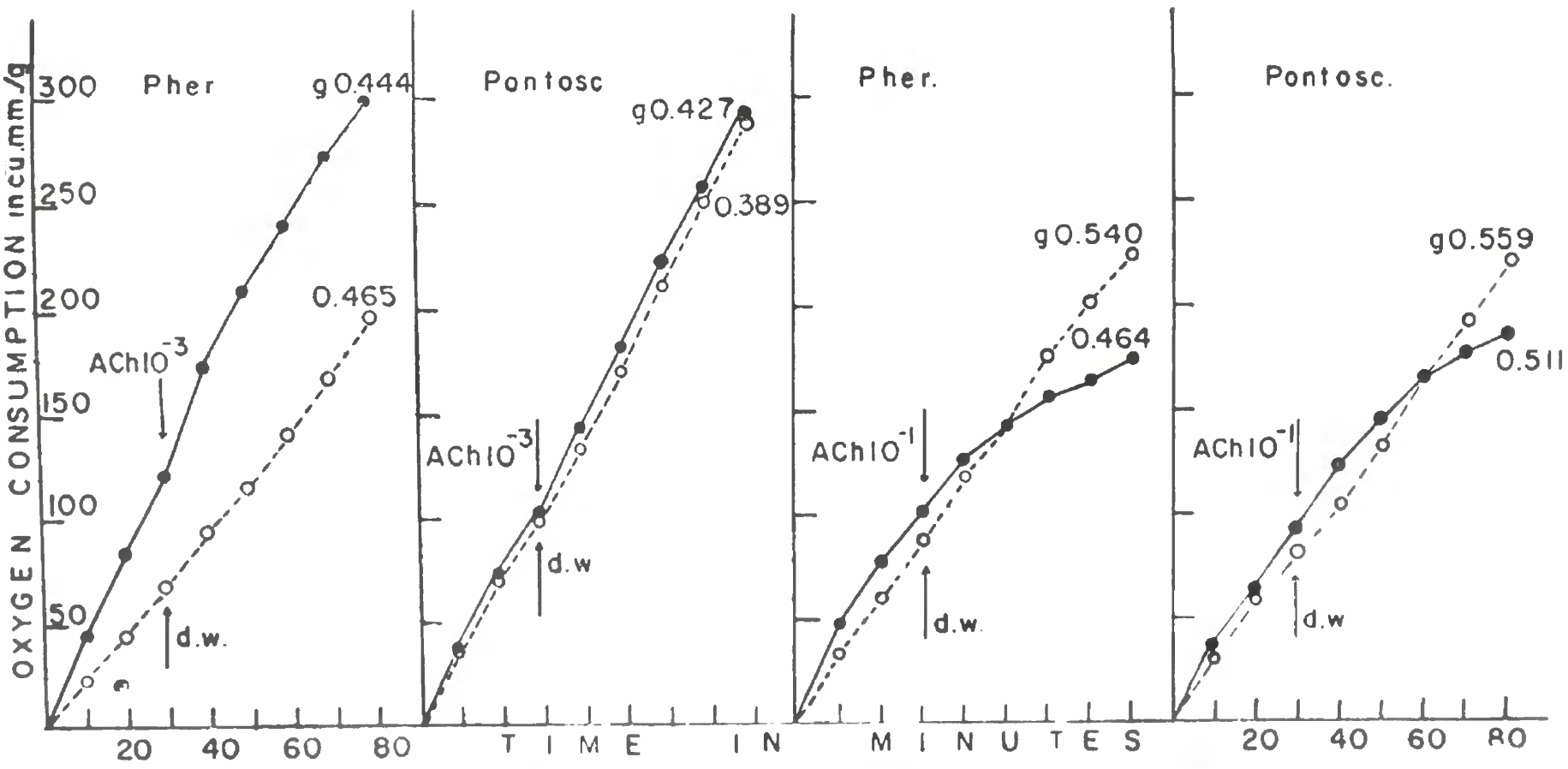


Fig. 7: The action of acetylcholine on the intraepidermal bloodvessels of the earthworms *Pheretima* and *Pontoscolex*, as judged from the modification of the respiratory rate. The arrows indicate when the drug was tipped on the animals.

Acetylcholine was also tested as 10^{-5}, 10^{-3} and 10^{-1} solutions. The graphs exposed in Fig. 7 are representative of the results generally obtained during the experiments with the two last concentrations. With 10^{-5} no changes were also observed in the respiratory rate. With 10^{-3}, as in the case of 10^{-3} Ad, it is hard to tell of any particular action of acetylcholine. With 10^{-1} a definite decrease in the oxygen consumption was observed, both in *Pheretima* and *Pontoscolex*.

The possibility of an acetylcholine breakdown by the mucous expelled by the animals was also checked, using a technique similart to that of AMMON (1934). In a first series of experiments, intact living animals were put in the vessels in atmosphere of 5% CO_2 in nitrogen and from the sidebulbs a 10^{-3} acetylcholine solution in bicarbonated saline (PROSSER & ZIMMMERMANN 1943, p. 78) was added to the main chamber. The results, although irregular, were never in the sense of a definite CO_2 production inside the vessels. Rather, gas absorption took place in some cases. In a second series of experiments, mucous was extracted from the animals with ether and dissolved in the main chamber. No CO_2 production was also observed in this series.

DISCUSSION

a. The penetration of an epithelial tissue by blood vessels, according to STEPHENSON (l. c.), is extremely rare. Besides the mentioned occurrences in Oligochetes, capillaries are also found in the epidermis of leeches and apparently in the ear of alligators and less clearly in the ear of rabbit and of man and in the olfactory mucous membrane of the guinea-pig (LENHOSSEK l. c.). From a list of SOUZA & SAWAYA (1928), however, it seems that the occurrence of intraepithelial capillaries is not so rare, since it was observed in many regions of the human body (in the male urethra by SOUZA & SAWAYA l. c.), in many other mammals, in birds, reptiles, amphibians and fishes. A great number of these alleged cases of penetration os blood vessels in epithelial tissues, however, are critized on the ground that due to poor histological technique the subcutaneous vessels were shifted into the epithelial layer and taken as intraepithelial. On the other hand, it is true that for ma-

ny of the reported occurrences of intraepithelial blood vessels it is hard to find a physiological meaning. In those cases, however, where it is possible to correlate the presence of intraepithelial capillaries with the fact that the regions where they occur serve to respiration, this peculiar position of blood vessels acquires an obvious physiological meaning. This situation is found in many fishes, such as *Cobitis* (LORENT 1878, apud WINTERSTEIN 1921, p. 145) and amphibians, such as Gymnophiona (SARASIN 1887, FURHMANN 1912, MENDES 1941 and GRINKRAUT 1949), Urodela (especially BETHGE 1897) and Anura (LEYDIG 1872, apud WINTERSTEIN l. c., p. 192, NOBLE 1925), where respiration through an epithelial layer facilitated by the presence of sub-or-intraepithelial capillaries is considered as an accessory or even, as in the case of the lungless Amphibians, the main respiratory process. The Oligochetes, in this respect, surely resemble the lungless Amphibians. Their lack of special respiratory organs is compensated for by the utilization of the entire skin as a sort of branchial organ and the efficiency of the skin as a respiratory organ obviously must be largely increased by the penetration of the capillaries into the outermost layers, as BEDDARD (l. c., p. 76) already recognized. In tropical earthworms such as *Glossoscolex sp.*, *Pontoscolex sp.* and *Pheretima hawayana*, which often are exposed to low tension of oxygen in the air of their burrows, the intraepidermal disposition of capillaries is certainly a great help in reckoning with such a difficulty.

The fact that in the Megascolecida *Pheretima hawayana* the intraepidermal loops are single, in the two genera of Glossoscolecidae (*Glossoscolex* and *Pontoscolex*) they are double and, finally, that in the Lumbricida *Lumbricus* the two types of loops are found, kept us wondering whether or not a correlation exists between the shape of these vessels and the position in the system, or, even, the mode of living. Unfortunately, due to the lack of information as to the shape of the intraepidermal capillaries in the majority of the reported occurrences, little can be advanced here with regard to this question. Capillaries ending blindly were mentioned in the epidermis of a *Microdrilum*, the Tubificida *Limnodrilus hoffmeisteri* by STEPHENSON (l. c., p. 183). MARCUS (l. c., p. 180).

however, could not confirm their existence. Besides *L. hoffmeisteri* was in 1935 identified with *L. socialis* (*gotoi*) by MICHAELSEN, where NOMURA (l. c., p. 25) described the vessels as forming loops. On the other end of the system, we find in the Lumbricida *Allolobophora dubiosa* a high degree of complexity of the intraepidermal capillaries. According to SZÜTS (l. c.) in this Oligochete the loops form complicating branchings of which "baskets" of capillaries are produced. These capillaries, as SZÜTS expresses it, strive to spread themselves out over as large a surface as possible, a disposition which is correlated by the author with the aquatic habit of the worm.

b) To our knowledge no experiments exist performed with substances affecting the capillaries in order to find out whether or not, by altering the caliber of sub-or-intraepithelial vessels in presumably respiratory surfaces, a modification of the respiratory rate would occur.

In dealing, however, with the external application of drugs, the question arises of whether the substances really permeated through the skin and reached the capillaries. In Crustaceans (PROSSER 1941, p. 1146) it was possible to make acetylcholine act upon the heart of the crayfish from a 10^{-4} solution, which entered the body by the gills alone. In our earthworms, from the position occupied by the capillaries in the epidermis, we think that the chances of the drugs having reached them were good, especially when strong concentrations were used. The only objection against this view derives from a possible barrier which the mucous expelled by the animals, as a reaction to the sudden contact with the fluids tipped from the sidebulbs, would oppose to the penetration of the drugs. This mucous may have acted as a physical barrier or, even, by neutralizing the action of the substances. This would also explain why only with very strong concentrations, such as 10^{-1}, definite effects of acetylcholine and adrenalin were observed. The possibility of an acetylcholine breakdown, however, by the mucous alone was dismissed by the absence of any cholinesterase powers revealed by the tests.

Adrenalin and acetylcholine, when used in concentrations strong enough (10^{-1}) to overcome the mucous barrier, caused respective-

ly and increase and a decrease of the respiratory rate. The observed effects canot be atrributed to modifications of the muscular activity of the animals. After the first contact with the sidebulb contents, whether distiled water or drug solution in any of the concentrations used, the animals would become a little agitated and then stay quiet in the vessels with occasional crawlings. We, therefore, are inclined to admit that the effects observed were possibly due to drug action upon the capillaries, from which resulted modifications of the blood flow and ultimately of the oxygen uptake. From the observed increase of the respiratory rate obtained with 10^{-1} Ad, the drug might be considered as producing vasodilatation in earthworms. From the decrease obtained with 10^{-1} ACh, constriction power might be attributed to this drug. This is the reverse of what is observed in the Vertebrates. This reverse action of adrenaline and acetylcholine upon the Oligochetes effectors was, in a way, also observed in the case of the heart, where PROSSER & ZIMMERMANN (l. c.) found that acetylcholine can stop in systole the hearts of *Arenicola* and *Lumbricus* (it blocks the frog heart in diastole) whereas, at least in *Arenicola,* adrenaline arrests the heart in diastole (it stops the frog heart in systole).

SUMMARY

1. Studies on the cutaneous respiration of one Megascolecida (*Pheretima*) and two Glossoscolecidae (*Glossoscolex* and *Pontoscolex*) were performed in order to find out whether, in these earthworms, intraepidermal capillaries occurred or not and, in the case of a positive answer whether or not these capillaries might be influenced by drugs having a known effect in other capillaries.

2. Intraepidermal capillaries exist in the three earthworms studied. In *Pheretima* they have the shape of single loops (table I, fig. 5), whereas in *Glossoscolex* and *Pontoscolex,* the intraepidermal loops are double (Table I, figs. 1, 2, 3 and 4).

3. Attention is called to the fact the shape of the capillaries seems to be a constant of the families: the Megascolecida *Pheretima* with single intraepidermal loops, the Glossoscolecidae *Glossoscolex* and *Pontoscolex* with double loops and the Lumbricida *Lum-*

bricus (LENHOSSEK l. c.) with single and double loops. The necessity of more detailed information on the shape of the intra-epidermal capillaries in Oligochetes is stressed in order to establish possible phylogenetical and ecological correlations.

4. When used in concentrations strong enough to overcome the mucous barrier opposed by the animals (10^{-1} solutions) adrenalin and acetylcholine caused respectively an increase and a decrease of the respiratory rate.

5. No cholinesterase activity was detected in the mucous extracts.

6. From the increase of the respiration obtained with adrenalin, the assumption is made that under the action of the drug a larger flow of blood went through the capillaries, as a consequence of their dilatation. From the decrease of respiration, on the other hand, observed with acetylcholine, a constriction of the capillaries is suggested under the action of the drug, which resulted in a smaller blood flow.

7. These action of adrenalin and acetylcholine upon capillaries is the inverse of what is observed in vertebrates, for instances, in the frog. The fact that acetylcholine causes arrest in systole of the hearts of *Arenicola* and *Lumbricus* and, in *Arenicola* at least, adrenalin causes heart blocks in diastole, is recalled.

SUMÁRIO

1. Foram estudados alguns aspectos da respiração cutânea de um Megascolecideo (*Pheretima*) e dois Glossoscolecideos (*Glossoscolex* e *Pontoscolex*), a saber, a existência nesses 3 oligoquetos terrestres tropicais de capilares intraepidérmicos tais como foram descritos em *Lumbricus* por LENHOSSEK (1895) e, no caso de uma resposta positiva, se êsses capilares poderiam ser afetados por drogas que têm uma ação conhecida sôbre outros capilares.

2. Capilares intraepidérmicos existem nos 3 vermes estudados. Em *Pheretima*, têm êles a forma de uma alça simples (Fig. 5 da tab. I), enquanto que em *Glossoscolex* e *Pontoscolex* as alças intraepidérmicas são duplas (Figs. 1, 2, 3 e 4 da tab. 1).

3. Chama-se a atenção para o fato de que a forma dos capilares parece ser uma constante das famílias: O Megascolecideo

Pheretima com alças simples, os Glossoscolecideos *Glossoscolex* e *Pontoscolex* com alças duplas e o Lumbricideo *Lumbricus* (LENHOSSEK l. c.) com alças duplas e simples. Acentua-se a necessidade de estudos mais detalhados sôbre a forma dos capilares intraepidérmicos nos Oligoquetos para as correlações filogenéticas e ecológicas.

4. Quando empregadas em concentração suficientemente forte para vencer a barreira de muco expelida pelos animais (10^{-1}), adrenalina e acetilcolina causaram respectivamente um aumento e uma diminuição da taxa respiratória em *Pheretima* e *Pontoscolex*.

5. Não foi observada atividade colinesterásica em extratos de muco.

6. Do aumento respiratório obtido com adrenalina, pressupõe-se que sob a ação da droga ocorreu um maior fluxo de sangue pelos capilares intraepidérmicos, como conseqüência de sua dilatação. Da diminuição observada com a acetilcolina, sugere-se uma vaso-constrição dos elementos situados intraepidermicamente, de que resultou menor afluxo de sangue.

7. Essas ações da adrenalina e da acetilcolina sôbre capilares é o inverso do que se observa nos vertebrados, p. ex., na rã. De um certo modo, essa inversão de ação das duas drogas também se observa em outro efetuador dos Oligoquetos, a saber, o coração. Aqui, PROSSER & ZIMMERMANN (1943) demonstraram que acetilcolina pode parar em sistole o coração de *Arenicola* e *Lumbricus* (ela bloqueia o coração da rã em diastole), enquanto que a adrenalina, pelo menos em *Arenicola*, para o coração em diastole (na rã o coração é detido em sistole).

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