SOMMAIRE

TROIS ESPÈCES NOUVELLES DE *PROCAMPYLASPIS* (CUMACEA) DES EAUX DE LA MAURITANIE (ATLANTIQUE TROPICAL DE L'EST)

PAR

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The authors, by studying a material collected on the "Thalassa" vessel in the waters of western tropical Africa (the Mauritanian coast), describe three new species belonging to the *Procampylaspis* genus : *Procampylaspis arguini*, *Procampylaspis thalassae* and *Procampylaspis maurini*.

Par l'amabilité du Prof. Claude Maurin, Directeur de l'Institut Scientifique et Technique des Pêches Maritimes de France, l'un d'entre nous (Băcescu) a eu la chance de participer activement à la troisième Campagne de la Thalassa dans les eaux de l'Afrique Nord-occidentale, en janvier-février 1971. Au cours de la Campagne dirigée par le Dr Marc Bonnet, Directeur de la Station de Sète, on a effectué 156 opérations entre le Cap Juby et Mottes d'Angel [1] ; les 32 dragages et 15 analyses du contenu de la sonde atachée au chalut ont été effectués sous la responsabilité de M. Băcescu. Les dragages ont été exécutés depuis 10 m à 1100 m de profondeur dans l'espace mentionné. On y a capturé ainsi, entre autres, des centaines d'exemplaires de Cumacés.

Nous disposons d'un nombre réduit de travaux sur les Cumacés de l'Afrique tropicale occidentale, dont les plus consistants sont ceux appartenant à N.S. Jones (1956— 1959). Ses études portent surtout sur les Cumacés de l'infralittoral (entre 6—50 m) ; il n'a eu à sa disposition que 2 échantillons plus profonds (108 et 220 m).

Ceci explique l'absence totale des représentants du genre *Procampylaspis* et la citation d'un seul *Campylaspis* (*C. glabra* Sars) dans ses travaux, tandis que le matériel capturé par nous présente 3 espèces nouvelles du premier genre et 4—5 du second, rien que du côté de la Mauritanie.

En tout état de cause, si pour les eaux de la côte du Sénégal, on connaît 20 espèces de Cumacés, au long de la Mauritanie on ne connaissait jusqu'à présent qu'une seule espèce (*Eocuma calmani*) décrite par Fage [4].

Dans la note présente nous décrivons 3 espèces nouvelles de *Procampylaspis* trouvées dans les eaux de la Mauritanie, à savoir :

1. Procampylaspis arguini n. sp.

(fig. 1 et 2)

Description. ♀ P. Le tégument dans son entier, très fin, non calcifié, sans écailles et sans adhérences de vase ou de sable, malgré les soies longues et relativement épaisses, qu'on voit partout. La *carapace* bombée, sans tubercules, gibosités ou plis ; le rapport longueur/largeur/hauteur est : 14 : 9,5 : 7,5 (fig. 1 A). De profil, une légère encoche antennaire et le bord inférieur lisse (fig. 1 B). Lobe oculaire totalement absent. Pseudo-rostre court, légèrement redressé, à faible encoche médiane et frontale sur chaque moitié. Les thoracomères, vus en dessus, sont en général cachés par la carapace. Seul le premier segment thoracique libre possède un sillon médian dorsal séparant deux lamines arquées en denticules.

Maxillipède I (fig. 1 C) présente un dactylopodite allongé et rétréci au milieu et l'endite qui porte les retinacles finit par une épine et quelques soies, dont une très forte, barbelée. Le dactyle falciforme du maxillipède II, si caractéristique du genre, présente ici deux longs prolongements dactyloïdes entre lesquesls une courte épine et une apophyse lamellaire proximale qui porte une dent antérieure (fig. 1 D et E). La partie courbée du dactyle, légèrement gauffrée. Les autres articles du maxillipède II ont des phanères usuels (fig. 1 D). Le maxillipède III (fig. 1 F), les péréiopodes II (fig. 2 B) n'ont pas une morphologie particulière ; nous mentionnons un ischium clairement individualisé pour le péréiopode II. Les carpo-méro- et ischiopodites du premier péréiopode ont des tailles inégales, décroissant en longueur depuis le propode à l'ischiopodite (fig. 2 A) ; le flagellum de l'exopodite présente seulement 3 articles (fig. 2 a) tant ici que chez le péréiopode II.

L'abdomen, quelque peu plus court que la carapace, sans tubercules ou écailles, mais avec de longues soies simples, latérales, ayant le dernier article légèrement bombé.

Les uropodes (fig. 2 C) presque aussi longs que l'ensemble des 4 derniers segments abdominaux ; leur basis plus long que les 2 derniers pléonites, presque 2 fois plus long que son endopodite et bien fin (rapport longueur/largeur : 8 : 1). L'endopodite plus long que l'exopodite, avec les habituels 3 phanères internes à soies courtes et un phanère distal du même type, mais beaucoup plus fort, à court flagelle subterminal. L'exopodite avec sa longue épine terminale non flagellée atteint le niveau distal de l'endopodite. Couleur ivoire sans taches.
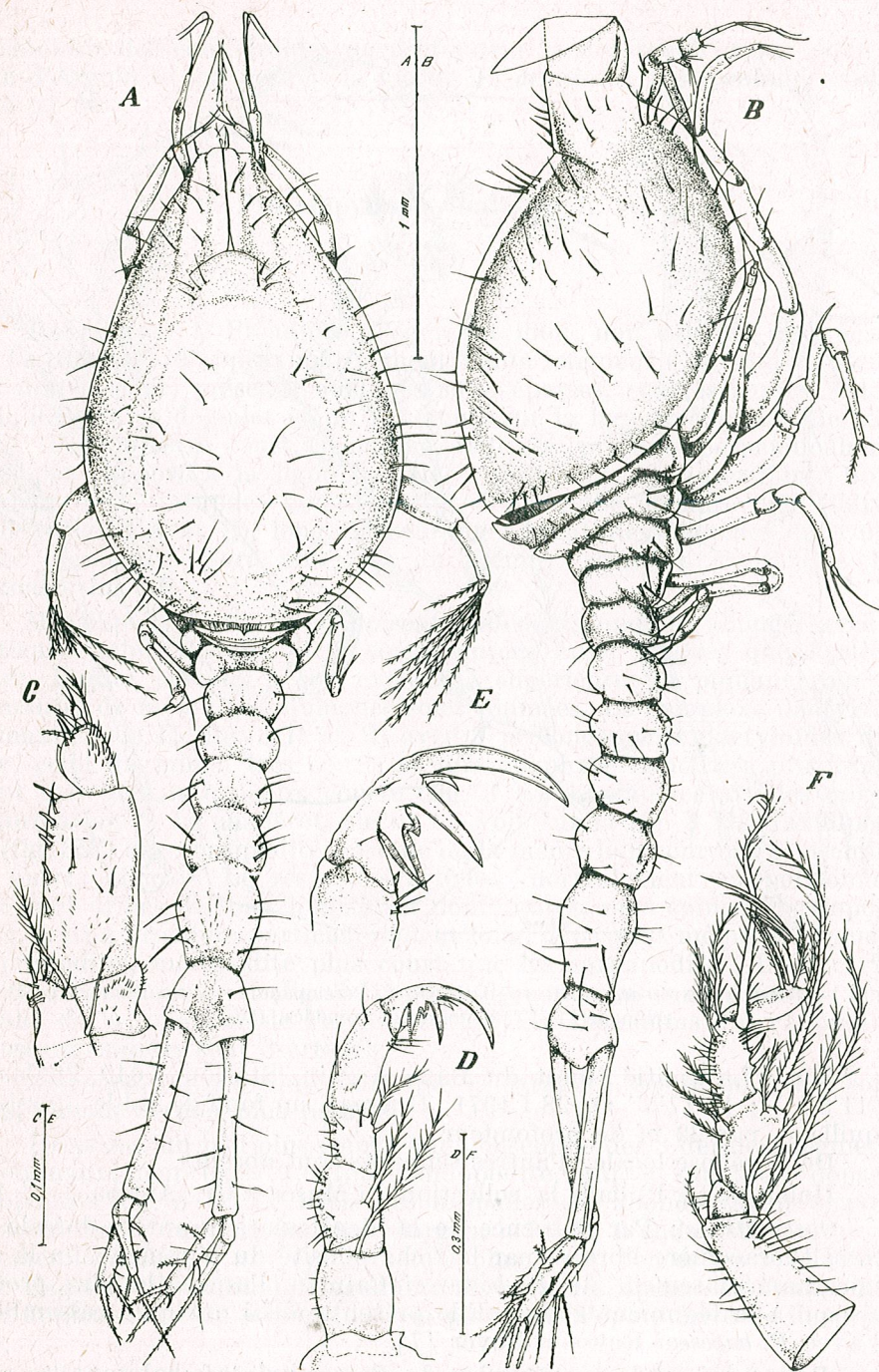
Longueur = 4 mm (♀ préadulte).

Fig. 1. — *Procampylaspis arguini* n. sp. ♀ juv. : A et B, ♀ vue d'en haut et de profil ; C, Maxillipède I ; D, Maxillipède II ; E, son dactyle falciforme, grossi ; F, Maxillipède III. (Orig.)
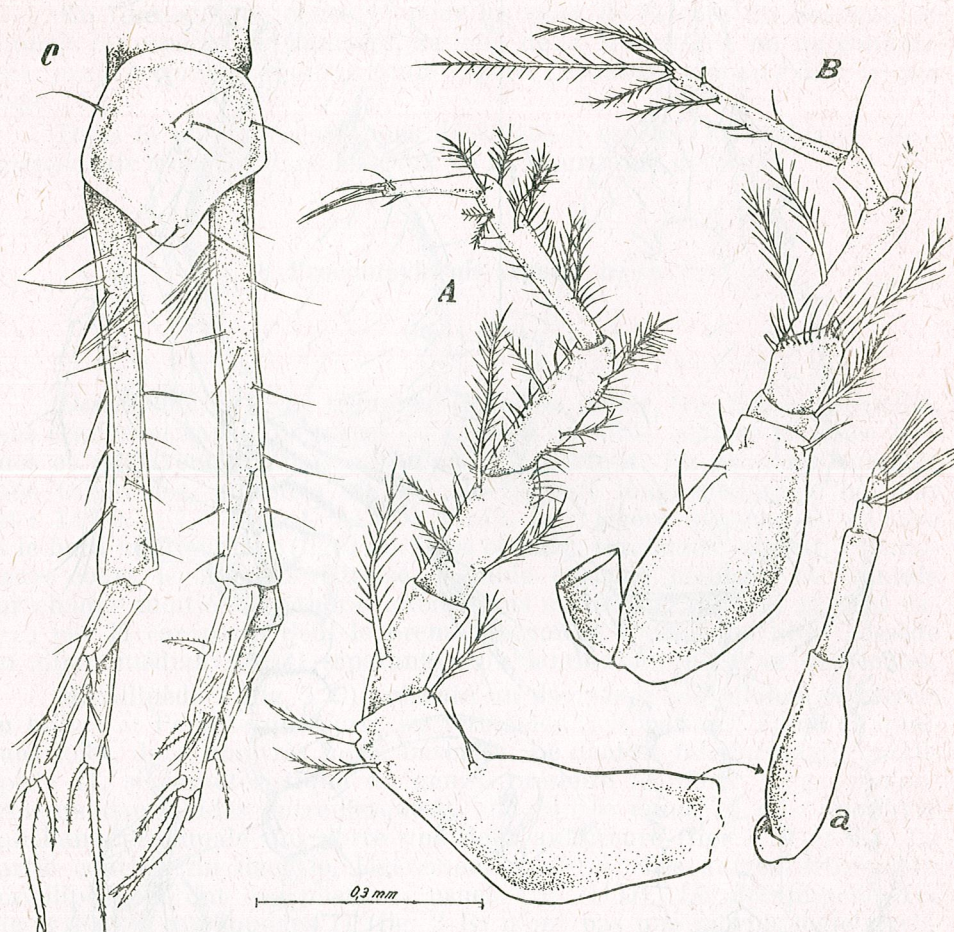
Fig. 2. — *Procampylaspis arguini* n. sp. ♀ juv.: A, Péréiopode I; a, son exopodite; B, Péréiopode II; C, pléotelson et uropodes. (Orig.)

Loc.: La moitié Ouest du Banc Arguin, Station X040 Thalassa: 20°11′3″ N; 17°17′5″ O, 28.I.1971. Dragage sur fond de sable grossier à coquillage, par 22 m de profondeur.

De la cénose locale, d'autres Cumacés sont absents.

Holotype (♀ P) dans la collection du Musée « Gr. Antipa », n° 172.

Observations: Par l'absence de la denticulation antéro-dorsale du second thoracomère libre et par la riche pilosité du tégument, on la distingue immédiatement de *P. bonnieri* Calman, l'espèce la plus proche, mais qui a le tégument glabre. Elle présente aussi quelques ressemblances avec *P. bacescoi* Reyss et Soyer [7].

P. arguini est la seule espèce de *Procampylaspis* habitant les eaux de l'infralittoral. En effet, seulement *P. sordida* Hale et *P. armata* Bonnier montent jusqu'à 60 m., respectivement 200 m de profondeur du circalittoral, donc jusqu'à la limite supérieure du talus continental. C'est

la remontée des eaux froides de profondeur — si caractéristique pour le Banc d'Arguin — qui permet la vie de *P. arguini* à une profondeur d'à peine 22 m.

2. Procampylaspis thalassae n. sp.

(fig. 3)

Description. ♀ Préadulte. Tégument mou, non calcifié, sans écailles. *La carapace* (d'approximativement 1 mm longueur 0,55 mm hauteur, 0,63 mm largeur) présente quelques soies éparses, courtes sur les côtés, quelques touffes dorsales et un tubercule sur la ligne médio-dorsale (fig. 3 A). Pseudorostre court, accuminé, redressé presque perpendiculairement par rapport à la ligne dorsale. Faible encoche antennaire. Bord inférieur de la carapace non armé (fig. 3 A). Lobe oculaire bien individualisé (fig. 3 A et B), long, présentant 4 omatidies blanches dans une masse pigmentée. Bord antérieur du premier segment thoracique bidenticulé (fig. 3B).

Maxillipède I (fig. 3 C) présente un dactylopodite allongé, avec 2 denticules inféro-distaux et 2 soies simples aussi longues que l'article entier. Endite portant à son extrémité supérieure un piquant robuste et hirsute, de même que quelques soies plumées. Le complexe dactylaire du maxillipède II (fig. 3 D et E) avec 3 prolongements dactyloïdes distaux, celui du milieu très court, et une apophyse lamellaire proximale, ayant une dent antérieure, courte (fig. 3 E). Les autres articles ont la conformation et la phanérotaxie qu'on voit sur la fig. 3 D. Maxillipède III (fig. 3 F) avec basipodite presque de la même longueur que l'ensemble des autres articles. Le reste des articles, normalement proportionnés, fortement armés de soies longues et nombreuses, sans épines. Péréiopode I (fig. 3 G) a l'ischium particulièrement long, dépassant même la longueur du propodite; méropodite plus court que les carpopodites. Le dactylopodite du péréiopode II (fig. 3 H) porte de nombreuses soies fortes; celle distale, de la longueur de l'article entier. Les flagellums des exopodites de ces appendices sont 3-articlés.

Abdomen, un peu plus court que la carapace, ses pléomères à soies rares, l'avant-dernier plus renflé.

Uropodes, un peu plus courts que l'ensemble des 3 derniers segments abdominaux, leur basis 1,6 fois l'endopodite, qui, à son tour, dépasse l'exopodite (fig. 3 I). Le basis des uropodes est abondamment couvert de soies fines; rapport longueur/largeur: 7,5/1. L'endopodite armé de 3 épines latérales et une épine distale plus forte, toutes les 4, plumées, avec flagelle subterminal; flagellums absents des épines de l'exopodite, dont l'apicale atteint le niveau de la pointe de l'épine forte de l'endopodite.

Coloris: blanc-ivoire, à teinte jaune pâle.

Appendices abondamment encrassés de vase.
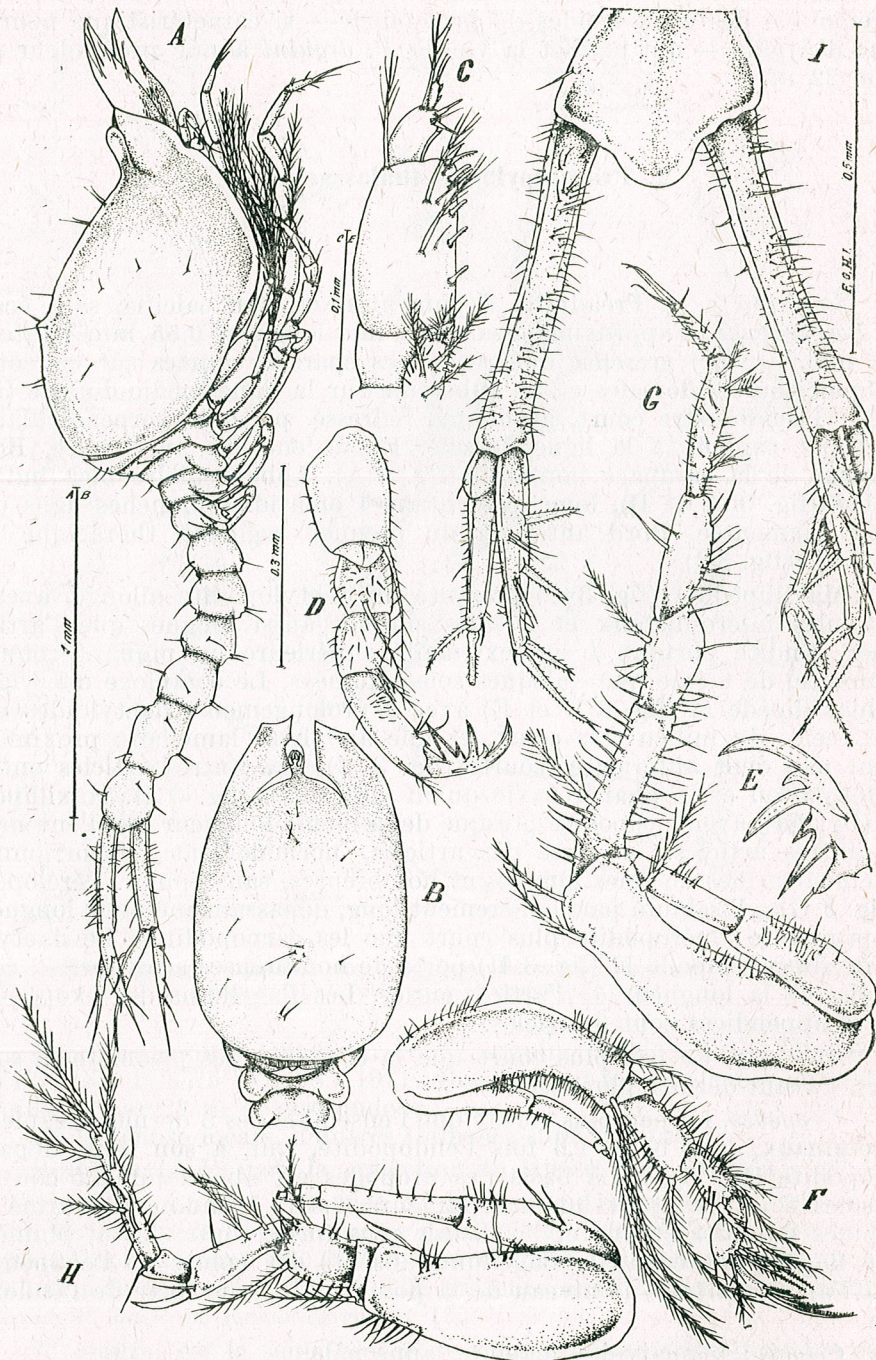
Taille ♀ P: 2,74 mm.

Fig. 3. — *Procampylaspis thalassae* n. sp. ♀ P : A, de profil ; B, Céphalothorax vu d'en haut ; C, Maxillipède I ; D, Maxillipède II ; E, son complexe dactylaire, grossi ; F, Maxillipède III ; G, Péréiopode I ; H, Péréiopode II ; I, Uropodes. (Orig.)

Loc. : Mauritanie, St. X048 : 28°50′5″ N ; 17°39′0″ O, 270 m, 4 ♀♀j, sur fond sable-vaseux ; St. X072 : 24°27′9″ N ; 16°26′0″ O, 286—227 m, 1♀ P

Holotype : 1 ♀ P, collection du Musée « Gr. Antipa », No. 173 + 1 Paratype ♀ j id.

Observations : *P. thalassae* appartient au groupe des six espèces ayant un lobe oculaire ; elle en diffère par sa forme globuleuse sans aucune expansion terminale et par la présence de deux paires de lentilles ocellaires virguliformes. *P. armata* sensu Bonnier (2) et *P. tridentatus Stebbing* ont chacune sur le lobe oculaire deux petits tubercules. *P. bacescoi* et *P. bituberculata Hansen* ont un lobe triangulaire et *P. sordida*, un lobe étroit, sans tubercules.

Parmi les espèces connues elle est plus proche de *P. armata* Bonnier (nec syn. *P. echinata* sensu Calman, **3**) mais celui-là diffère nettement par la structure du complexe dactylaire du maxillipède II dont les expansions lamellaires ont presque la même longueur, pour ne plus mentionner le lobe oculaire bifide.

3. **Procampylaspis maurini** n. sp.

(fig. 4 et 5)

Description. 1 ♀ marsupiphore. Tégument calcifié. *Carapace* à alvéoles petits, denses, lui prêtant l'aspect d'un rayon de miel (fig. 4 C), nombreuses irrégularités sur la surface ; elle est courte, porte des soies rares et deux tubercules d'une part et d'autre de la ligne médio-dorsale dans le tiers postérieur. Le bord inférieur de la *carapace présente une portion crénelée qui commence juste après la faible encoche antennaire* (fig. 4 B). Pseudorostre, long, presque le cinquième de la longueur totale de la carapace, fortement retroussé. Le lobe oculaire, absent. Les deux premiers segments thoraciques ont les bords antérieurs bidenticulés (fig. 4 C). A_2 a la forme qu'on voit sur la figure 4D.

Maxillipède I (fig. 4 F), caractérisé par son dernier article, court, globuleux, terminé par une soie longue, et par l'endite armé à son extrémité antérieure d'une épine glabre, forte, doublée d'une petite soie. Le complexe dactylaire du maxillipède II (fig. 4 G et I) porte 3 épines ou prolongements dactyloïdes (celui du milieu 2 fois plus petit) et 2 prolongements lamellaires triangulaires, proximaux, disposés sur un plan inférieur aux épines. Le basipodite à nombreuses écailles spiniformes et quelques groupes de spinules courtes sur la face extérieure, aussi très caractéristique de l'espèce (fig. 4 G et H). Le maxillipède III (fig. 5 A) aux bords inférieurs de l'ischiopodite et du méropodite (partiellement) armés de courtes spinules. Le péréiopode I (fig. 5 B) présente un propodus de taille presque égale à celle de l'ischiopodite, tandis que le carpopodite a une longueur égale au méropodite. Le péréiopode II (fig. 5 C) a un dactylopodite d'une longueur égale au carpopodite, le dernier armé de 2—3 spinules distales ; le flagelle de l'exopodite des péréiopodes présente 4 articles.
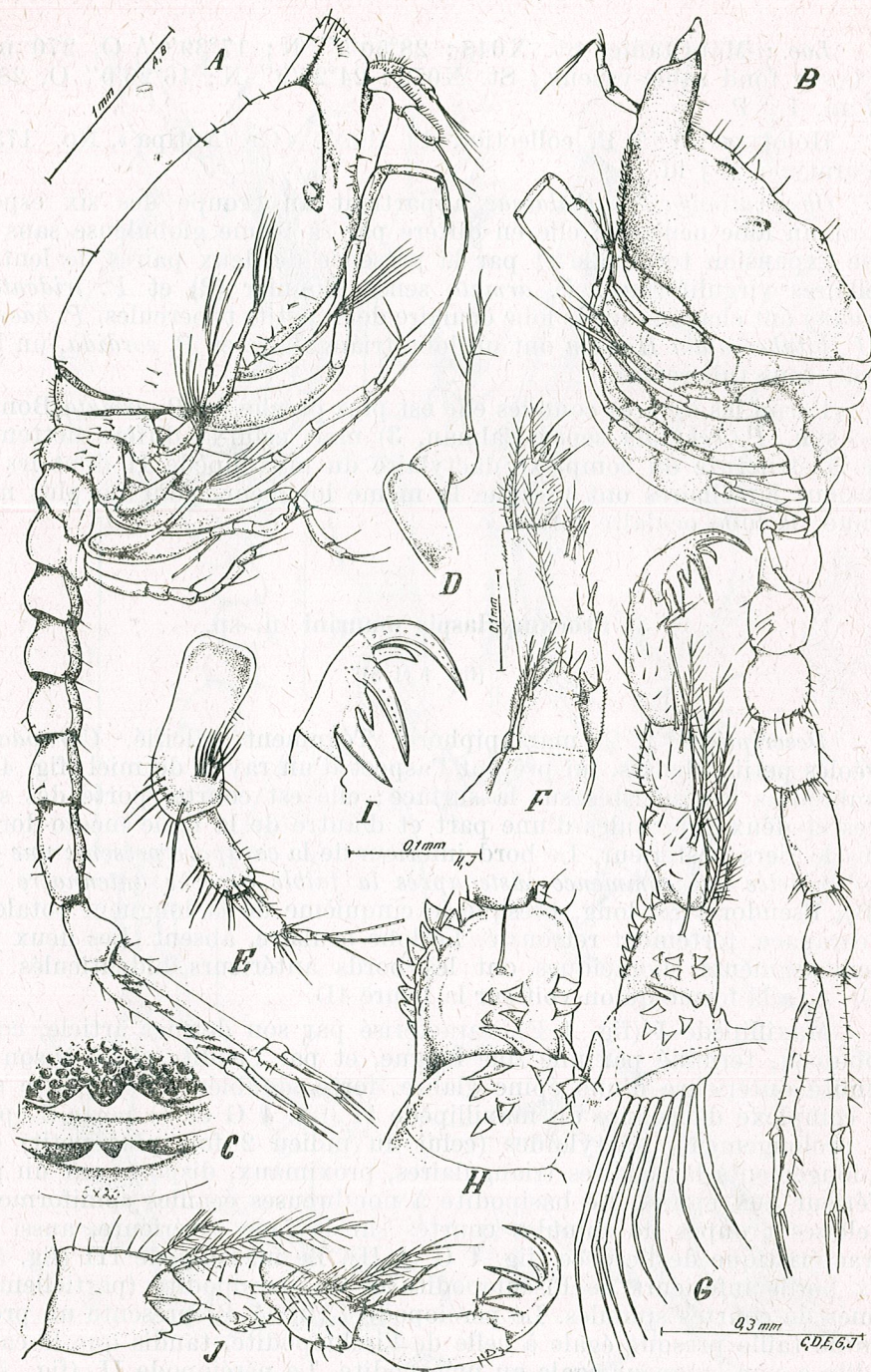
Fig. 4. — *Procampylaspis maurini* n. sp. ♀ M et ♂ N : A, ♂ N — de profil ; B, ♀ M — de profil ; C, aspect grossi du tégument de la carapace et de l'encoche des deux thoracomères libres (♀ M) ; D, Antenne II (♀ M): E, Antenne I (♂ j) ; F, Maxillipède I (♀ M) ; G, Maxillipède II (♀ M): H, son basipodite, grossi ; I, son dactylopodite, grossi ; J, Maxillipède II (♂ N). (Orig.)
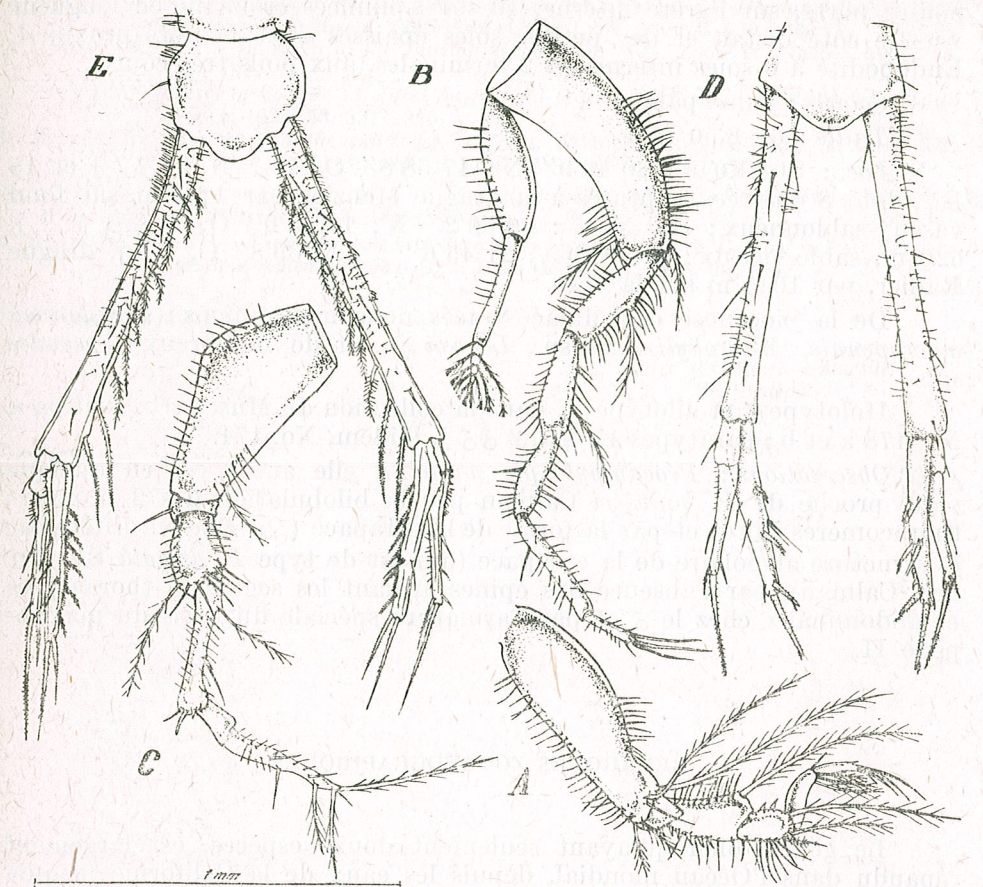
Fig. 5. — *Procampylaspis maurini* n. sp. ♀ M et ♂ N : A, Maxillipède III (♀ M) ; B, Péréiopode I (♀ M) ; C, Péréiopode II (♀ M) ; D, Uropodes (♀ M) ; E, Uropodes (♂ N). (Orig.)

Les uropodes (fig. 5 D) presque aussi longs que les 3 derniers segments abdominaux. La base, svelte ; l'endopodite à 3 épines latérales, en plus, une épine terminale, toutes plumées et avec un flagelle subterminal, lui-même hirsute. Son exopodite, un peu plus court, présente 3 soies légèrement plumées et une soie-épine très longue, sans flagelle subterminal (fig. 5 D).

2. ♂ N. *Carapace* sans gibosités, mais alvéolée comme chez la ♀, avec des soies rares, dont certaines forment une rangée parallèle au bord inférieur. L'arête du dernier, lisse (fig. 4 A) non denticulée comme chez la ♀. Encoche antennaire très prononcée. Pseudorostre comme chez la ♀. A₁ (fig. 4 E) et A₂ ne présentent aucune particularité. Lobe oculaire absent. Les 2 premiers segments thoraciques échancrés au bord antérieur.

Uropodes (fig. 5 E) sveltes, de la longueur de l'ensemble des 4 derniers segments abdominaux. La base, deux fois la dimension de l'endo-

podite, porte, sur l'arête interne, 10 soies plumées croissant en longueur
vers le côté distal, et de petites soies épaisses dans le tiers proximal.
Endopodite à 6 soies internes et 2 terminales, aux poils très courts.

Coloris : jaune pâle

Taille : 6—6,50 mm.

Loc. : St. X046 : 20°49′9″ N ; 17°58′8″ O, 1 ♂ N, 2 ♂♂ j et 15
♀♀, dont 8 œuvrées, capturés à la drague Menzies par 1120 m, sur fond
vaseux-sablonneux ; St. X047 : 20°50′2″ N ; 17°43′0″ O, 2 ♀♀, 1 ♂ j,
620 m, sable-vaseux ; St. XO55 : 21°45′6″ N ; 17°39′8″ O, 1 ♀ j, drague
Rallier, par 1045 m sur la vase.

De la biocénose des Malacostracés, nous mentionnons : *Pseudomma
macropennis*, *Makrokylindrus* sp., *Leucon* sp. et de nombreux *Apseudes*
sp.

Holotype ♂ et allotype ♀, dans la collection du Musée «Gr. Antipa»,
No. 173 a et b ; paratypes 15 ♀♀, 2 ♂♂ j, ibidem, No. 174.

Observations : *Procampylaspis maurini*, elle aussi, est en quelque
sorte proche de *P. bonnieri* Calman par la bilobulation des 2 premiers
thoracomères libres et par la forme de la carapace (♀). Elle en diffère par
la structure alvéolaire de la carapace (qui est de type *P. armata* Bonnier
nec. Calman), par l'absence des épines armant les segments thoraciques
et abdominaux chez le ♂ et par l'armature spéciale du basis du maxilli-
pède II.

REMARQUES ZOOGÉOGRAPHIQUES

Le genre, bien qu'ayant seulement douze espèces, est largement
répandu dans l'Océan mondial, depuis les eaux de la Californie jusqu'à
celles de l'Australie ; depuis l'Islande jusqu'à l'Océan Antarctique.

Les espèces de *Procampylaspis* décrites ci-dessus sont des présences
nouvelles sur toute la côte occidentale de l'Afrique. Les espèces les plus
proches au point de vue géographique sont *P. tridentatus* dans le Sud
de l'Afrique (les eaux du Natal) et *P. armata* Bonnier (Açores).

Nos espèces semblent avoir des affinités plus précises avec celles
nord-atlantiques-méditerranéennes. Ce secteur, prolongé maintenant sur
la côte de l'Afrique tropicale occidentale, paraît être le centre génétique
principal de ce genre, vu qu'il abrite neuf des douze espèces connues jus-
qu'à nos jours.

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NOUVELLES ESPÈCES DE CERATOPOGONIDAE (DIPTERA)

PAR

ANDRIANA DAMIAN-GEORGESCU

Les 4 espèces présentées dans ce travail ont été capturées dans un piège à lumière installé à Gura-Zlata, dans le massif Retezat (Carpates Méridionales). Pour la 5ᵉ espèce, *Culicoides setosus* Gutzevich, dont seule la femelle avait été décrite, nous donnons la description du mâle.

Culicoides setosus Gutzevich 1960

♂. Longueur de l'aile = 1,45 mm ; largeur = 0,60 mm ; costa = 0,88 mm ; indice C/L = 0,60.

Hypopygium (Fig. 1). Lamelle entaillée dans la partie médiane, bordée par deux lobes proéminents. Processus de la lamelle coniques, longs, divergents. Basistyle long, cylindrique, les apodèmes ventraux et dorsaux courts, simples. Style long, à base renflée, très mince dans la moitié distale. Corps de l'aedeagus triangulaire, les bras courts chitinisés. Paramères non soudés, la partie basale fortement chitinisée, formant un angle droit avec la partie médiane, amincis vers le sommet et ne dépassant pas en longueur le sommet de l'aedeagus. Membrane basale nue.

Cette espèce fut retrouvée dans toutes les prises recueillies entre mai et novembre (le maximum d'exemplaires aux mois de juillet et novembre).
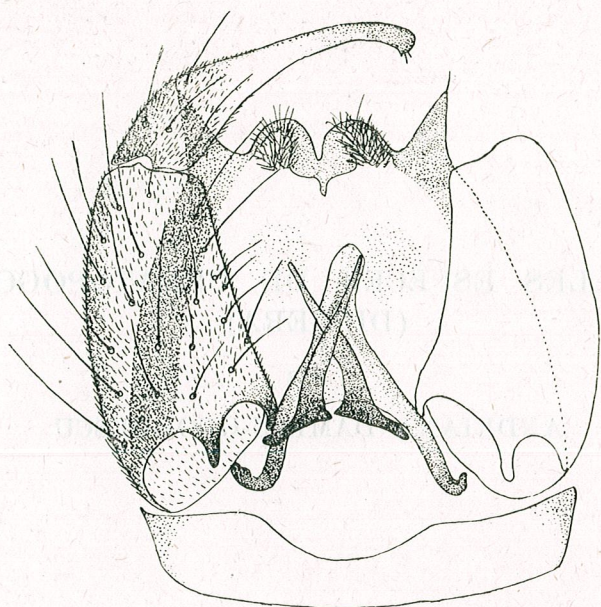
Fig. 1. — *Culicoides setosus* Gutzevich, hypopygium.

Culicoides remmi n. sp.

2 ♂♂ ont été capturés le 23 août 1970.

♂. Longueur de l'aile = 1,61 mm ; largeur = 0,59 mm ; costa = 0,96 mm ; indice C/L = 0,53.

L'aile (fig. 2b) tachetée avec la tache caractéristique en forme de clepsidre en r_5. Nervures très évidentes, bifurcation de la médiane (M) après la transversale (T). Le troisième article du palpe aussi large que les autres.

Hypopygium (Fig. 2c). La lamelle, fortement convexe, forme un lobe très allongé ; processus de la lamelle courts, mais bien individualisés, digitiformes, droits. Basistyles à base renflée, leurs bords internes peu convexes et munis de soies ; apodème ventral étroit, apodème dorsal massif, de la même longueur. Style peu renflé dans sa partie basale, l'extrémité distale élargie en bec de canard. Corps de l'aedeagus triangulaire, le sommet postérieur très aminci, à bout renflé en tête d'épingle (très ressemblant à celui de *C. moreli*, Clastrier) ; bras de l'aedeagus chitinisés. Paramères non soudés, leur partie basale formant un angle droit avec la partie médiane, amincis seulement dans le tiers distal. Membrane basale nue.
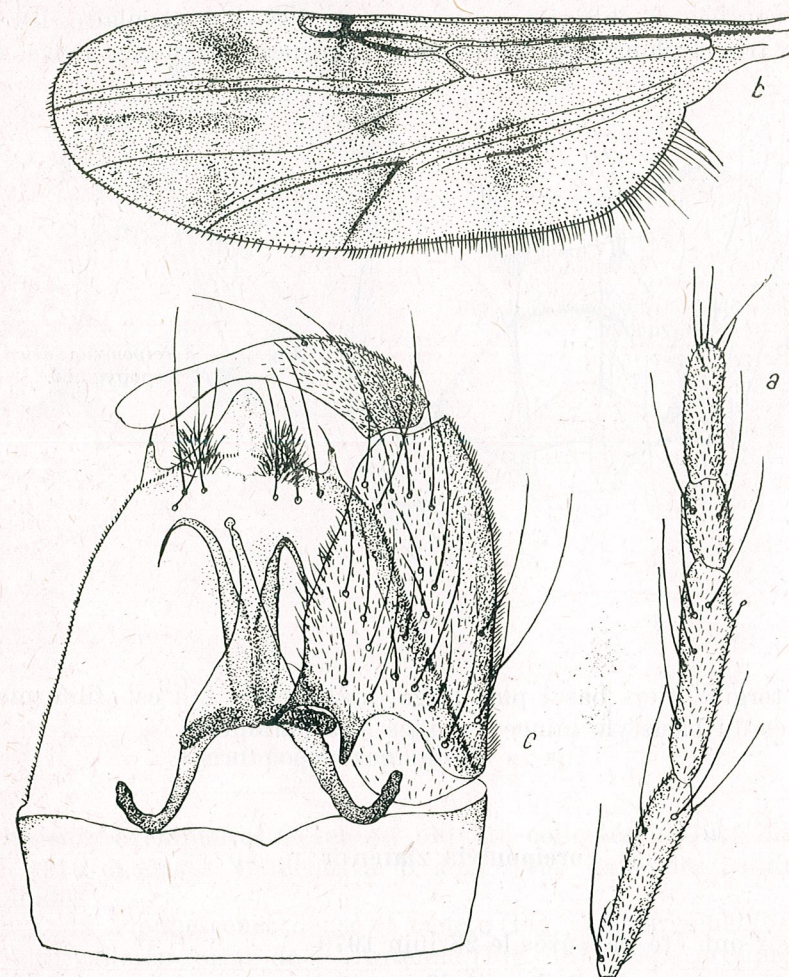
Fig. 2. — *Culicoides remmi* n. sp. ♂. a : palpe ; b, aile ; c, hypopygium.

Forcipomyia dacica n. sp.

3 ♂♂ ont été capturés le 19 mai 1970.

♂. Longueur de l'aile = 1,34 mm ; largeur = 0,46 mm ; costa = 0,57 mm ; indice C/L = 0,42. Aile sans taches, avec une seule cellule radiale (r_2) distincte.

Hypopygium (Fig. 3). Le 9e sternite petit, avec un petit lobe médian à bord concave ; le 9e tergite avec la partie basale fortement chitinisée, la partie distale peu chitinisée, avec un appendice proéminent muni aux coins postérieurs de 4 soies longues ; la longueur du tergite ne dépasse pas 2/3 de la longueur du basistyle. Basistyle court et épais, le bord in-

terne fortement convexe; style rétréci depuis la base au sommet, la pointe mince, courbée et chitinisée. Aedeagus de forme triangulaire, les angles latéraux très prolongés et modérément courbés. Paramères longs, dépas-
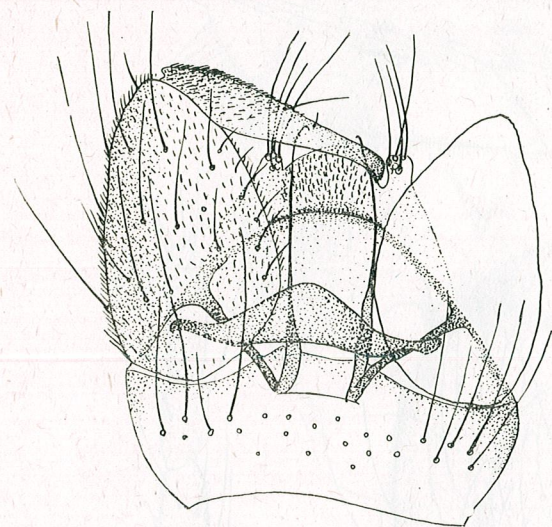
Fig. 3. — *Forcipomyia dacica* n. sp., hypopygium.

sant le tergite, tiers basal plus large que le reste qui est filiforme; les apodèmes du basistyle minces, soudés aux paramères.

Forcipomyia zlatensis n. sp.

2 ♂♂ ont été capturés le 23 juin 1970.

♂. Longueur de l'aile = 1,68 mm; largeur = 1,54 mm; costa = 0,80 mm; indice C/L = 0,47.

Hypopygium (Fig. 4). La partie basale du 9e tergite plus chitinisée que la partie distale qui présente de chaque côté un petit lobe muni de soies; le bord postérieur du tergite présente une petite fente et ne dépasse pas en longueur 2/3 du basistyle. Basistyle long et étroit, presque rectangulaire, style brusquement rétréci dans la moitié distale, pointe courbée et chitinisée. Aedeagus triangulaire, les angles latéraux fortement chitinisés, l'angle distal en forme de cône, étroit et long, de la même longueur que le corps de l'aedeagus, sa pointe aiguisée et chitinisée. Paramères soudés à la base en une large partie commune; d'ici se détachent les deux parties distales, droite et gauche, sous la forme de deux tiges filiformes, dépassant un peu le bord postérieur du 9e tergite. Les apodèmes longs et étroits touchent la base des paramères.
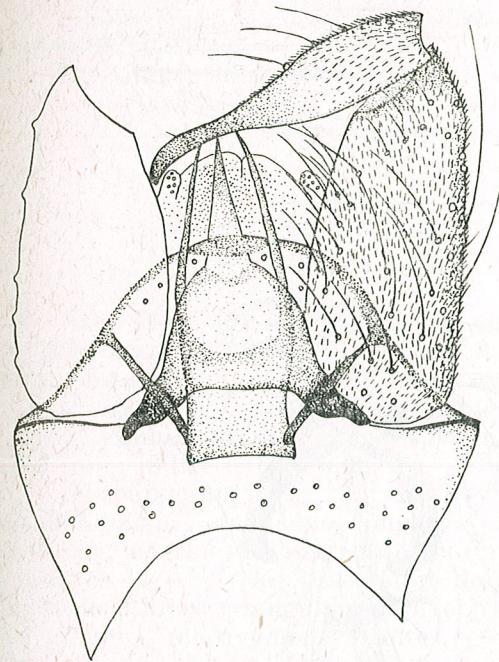
Fig. 4. — *Forcipomyia zlatensis* n. sp., hypopygium.

Ceratopogon romanicus n. sp.

Plusieurs exemplaires ♀♀ et ♂♂ ont été collectés à Gura-Zlata le 20 juin 1970 et au lac Gemene, le 3 août 1969 dans des essaims de Chironomides.

♀. Yeux à pubescence dense et courte (Fig. 5a), largement séparés par un intervalle en forme de V. Antenne (Fig. 5b). Scape presque noir; les articles 3 — 10 du flagelle d'un brun foncé, 11 — 15 brun clair; le 3e article 2 fois plus long que large, 4 — 10 presque sphériques, 11 — 14 subcylindriques, 15 sans stylet. Tous ces articles sont entièrement recouverts d'une très courte pubescence et présentent à la base une verticille de soies longues; chacun des articles 3 — 10 porte une soie sensorielle longue, dépassant l'extrémité distale de l'article. Longueur de l'antenne = 624 μ; indice antennaire = 1,4.

Les dimensions des articles (μ):

	3	4	5	6	7	8	9	10	11	12	13	14	15
long.	62	31	28	28	28	28	26	26	60	60	68	78	101
larg.	36	34	31	31	28	28	26	28	28	28	28	28	26

Fig. 5. — *Ceratopogon romanicus* n. sp., ♀. a, vertex; b, palpe; c, antenne; d, aile; e, spermathèques; f (♂), hypopygium.

Palpe (Fig. 5c) brun foncé recouvert d'une pubescence abondante. Longueur = 247 μ.

Les dimensions des articles (μ) :

	1+2	3	4	5
long.	73	62	44	68
larg.	23	23	21	23

Aile (Fig. 5d) sans taches, les deux cellules radiales très distinctes (r_2 plus grande que r_1). Longueur de l'aile = 1,79 mm ; largeur = 0,78 mm ; costa = 1,20 mm ; indice C/L = 0,66.

3 spermathèques piriformes, inégales (Fig. 5c).

♂. Longueur de l'aile = 1,53 mm ; largeur = 0,54 mm ; costa = 0,96 mm ; indice C/L = 0,62.

Hypopygium (Fig. 5f). Basistyle globuleux, dépassant la largeur de l'abdomen ; apodèmes longs ; style robuste, aminci dans la partie médiane, courbé à l'extrémité et muni de quelques soies. Paramères soudés à la base ; les bras latéro-dorsaux longs ; dans la partie médio-ventrale 2 lobes triangulaires fortement chitinisés et deux tiges terminales longues, relativement étroites, dont l'apex est courbé en tête d'oiseau. Aedeagus aux bras séparés, un peu élargis dans la moitié distale.

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TYPES OF DISTRIBUTION PATTERN AMONG FRESH-WATER ANIMALS

BY

PETRU BĂNĂRESCU

Three main types of distribution pattern can be recognized among epigeous fresh-water animals : 1) Primary aquatic animals (fishes, superior crustaceans, molluscs) whose ranges depend mainly on river drainages ; 2) Inhabitants of temporary pools with restricted range (the most important group being the Anostraca) whose ranges depend on climatic zones ; 3) Insects and water mites, of terrestrial origin ; the range of the rheophilic ones depend mainly on mountain ranges. Many hypogeous animals, both of marine and of fresh-water origin, seem to have retained the range of colonisation of subterranean waters, in spite of the old age of many of them.

Fresh-water animals are offshoots either of marine (fishes, crustaceans, mussels, prosobranchiate snails, most groups of so-called worms) or of terrestrial animals (insects, water mites, eventually pulmonate snails and some families of oligochaets). The distribution of migratory (diadromous) and of sporadic, e.g. euryhaline species occasionally entering inland waters, as well as that of higher taxa (genera and families) including resident fresh-water species of recent marine origin reflects rather marine zoogeography. I recently pointed out this fact for primary marine fish families including strictly fresh-water genera and species [1]. The same is true for several higher taxa of invertebrates, e.g. for the two families of shrimps occurring mainly (Palaemonidae) or almost exclusively (Atyidae) in fresh waters. Both families have a circumtropical range, with radiations in the temperate zone, especially in the circummediterranean area. Among their genera, one can distinguish : Indo-West Pacific (*Caridina*, *Paratya* among Atyidae, *Leptocaris* among Palaemonidae), tropical American (*Ortmannia*, *Potimirim*, *Xiphocaris*, then *Pseudopalaemon*), West African (*Caridella* and four related genera, then *Desmocaris*) and

circummediterranean ones (*Dugastella, Troglocaris, Atyaëphyra,* then *Typhlocaris*).

While the general distribution of these groups of recent marine intruders in inland waters reflects marine zoogeography, the distribution of their single resident species and especially that of all old fresh-water groups (both primary aquatic and of terrestrial origin) reflects terrestrial zoogeography. In all comprehensive treatises of zoogeography inland water groups and species are dealt with together with terrestrial animals. De Lattin [17] is, according to my knowledge, the only author having devoted, in a general book of zoogeography, an independent section to the fresh-water fauna.

The zoogeography of fresh-water animals actually is similar, but not quite identical to that of terrestrial ones. One main feature of the distribution of terrestrial organisms, which was clearly shown firstly by Reinig [21] and Ekman [10] and emphasized in recent years mainly by De Lattin [16] [17] is the contrast between "arboreal", "eremial" and "oreo-tundral" fauna and flora. The pleistocene refugial areas were different for forest (arboreal) and steppic (eremial) faunas; these refugial areas became, in post-glacial times, dispersal centres. Woodlands and humid meadow areas are inhabited, at present, by a fauna quite distinct from that living in arid steppes. The subdivisions of the Holarctic region are, according to De Lattin [17] different for the arboreal and for the eremial fauna.

No such subdivision ("arboreal" and "eremial") can be recognized for the true fresh-water fauna, although several authors tried to ascribe the discontinuities of the aquatic Holarctic fauna to the arboreal (Europe — East Asia — Alleghanies) and the eremial type (Central Asia — central North America). Reinig [21] for ex. considers the discontinuity of *Polyodon* (eastern North America) - *Pseuphurus* (East Asia) as arboreal, that of *Scaphirhynchus* (eastern North America) - *Pseudoscaphirhynchus* (Central Asia) as eremial. Actually the ranges of *Polyodon* and of *Scaphirhynchus* are the same, corresponding neither to the Alleghany woodland area, nor to the Kansan or Texan arid zone, but to the Mississippi drainage.

Terrestrial fauna and flora of woodlands and that of arid steppes are rather sharply delimited from one another. In East Europe both are separated by a line reaching from east of the Urals to the Lower Danube; the Hungarian Puszta is a western enclave of steppes surrounded by woodlands. Yet the main rivers draining this area (Danube, Dniester, Dnieper, Don) and their tributaries are inhabited by the same fauna of fishes, molluscs and superior crustaceans, both in woodlands and in steppes; this fauna includes some peculiar species and even genera, not occurring in the woodlands belonging to the Baltic and the North Sea drainages.

De Lattin [17] was aware of the fact that fresh-water animals cannot be ascribed to the same biochores (arboreal, eremial, oreo-tundral) as the terrestrial fauna and proposed for them two other biochores: the limnic or lacustrin and the riverine one. I have another opinion in this problem. Very many species inhabiting standing waters, especially ponds and surface-lakes in flood-plains of larger rivers, such as the

Danube or the Volga, occur also in the lower reach of rivers. The ranges of many species living exclusively in standing water correspond to river drainages : e.g. the European mud minnow, *Umbra krameri*, is restricted to the Danube and Dniester drainages. Even pure lacustrine species, if not marine relicts, are usually offshoots of riverine animals, that is they reached the lakes through the tributary rivers.

According to my opinion, the following types of distribution pattern can be recognized :

1. EPIGEOUS FRESH-WATER ANIMALS

a) Primary aquatic animals without possibilities of passive dispersal and whose distribution corresponds to river drainages

This category includes fishes, molluscs and superior crustaceans (Decapoda, Peracarida and the few epigeous Anaspidacea), then some groups of so-called worms (mainly Triclada). Excepting the few euryhaline and migratory species, this group includes animals strictly confined to fresh water, having no possibility to disperse either through salt water or by land (excepting a few fresh-water crabs [6] and some snails). Their distribution is confined to river drainages and their only possibility to extend from one river drainage to another is by means of river captures[1].

The ranges of the animals belonging to this category depends thus mainly on river drainages. Of course, not all species are confined to a single drainage; many of them, especially in Europe, live in several adjacent rivers. There are strong similarities between the faunas of many adjacent river drainages, e.g. the Rhine, Weser and Elbe, then the Danube and Dniester in Europe, the Kura-Araxes and Sefid Rud in western Asia, the Mekong and the Menam in South-East Asia, etc. Evidently, most species do not live throughout the whole river drainage, but only in suitable biotopes. The fauna of many large river drainages includes rather many endemic species, but only few of them inhabit the suitable biotope throughout the whole drainage (e.g. *Gobio uranoscopus* and *Acerina schraetser* in the Danube); most endemics are restricted to a small area within a drainage, usually to the drainage of a single or of a few adjacent tributaries of the main river. There are for ex. in the Danube drainage four endemic species of fishes and two of snails with a quite restricted range; tens of species are endemic to a restricted area within the Yangtze drainage, tens of other species of fishes (minnows, darters), of crayfishes and of fresh-water mussels to a restricted area (one or a few tributaries) of the Mississippi drainage. A lot of species inhabit a small area within a river drainage and an adjacent area in a neighbouring drainage, indicating that a rather recent river capture occurred.

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[1] The connection between the lower courses of rivers in the area of the present continental shelf, which occurred repeatedly during the lowering of sea-level in the pleistocene glacial periods, favoured the dispersal of aquatic animals also through the lower courses.

These facts show that there is no perfect correspondence between river drainages and ranges of the species belonging to this zoogeographic category of fresh-water animals; nevertheless their *main* distribution pattern reflects present (or past) hydrography. The most important fresh-water fauna whose range does not correspond to present hydrography (but reflects recent river captures) is the High-Asian one, occurring in the Tarim drainage, Balkash, Issykkul lakes, Tibet, etc., then in the upper reaches of Syr- and Amu-Darja, of Hilmena, Indus, Ganges, Brahmaputra, Mekong, Yangtze and Hwangho.

There is a great similarity between the regional distribution of fresh-water fishes, molluscs and Malacostraca (mainly Decapoda, but also Amphipoda and Isopoda). In North America the Rocky Mountains mark a sharp delimitation between two quite distinct faunas, a much richer eastern and a very poor western one; on the contrary, the Alleghany mountains represent a minor zoogeographical division line: fishes, crayfishes, amphipods, aquatic molluscs are represented by closely related, in many cases even by identical species in the Mississippi drainage and in the rivers flowing into the Atlantic Ocean. In Europe a correspondence is noticed between the zoogeographical provinces delimited by Berg [2] for fishes and by Žadin [26] and Starobogatov [25] for molluscs. The last-named author delimited in East Asia a Chinese subregion of the Oriental region, including also the Song-Koi drainage in North Vietnam and the coastwise rivers from Central Vietnam; the malacofauna of this subregion bears strong similarities with that of the Amur-Japanese subregion. This point of view corresponds exactly to the distribution of primary fresh-water fishes, especially minnows and loaches: the subfamilies Gobioninae, Xenocyprininae, Acheilognathinae, then many genera belonging to other subfamilies of minnows (Cultrinae, Danioninae, Cyprininae, even Barbinae) and to loaches, have an East Asian range, extending from North China (or even from the Amur and Japan) to North and Central Vietnam, but none of them, or only quite few, reach the Mekong drainage.

The same parallelism between the distributions of these three groups of primary aquatic animals occurs in South-East Asia, in Africa, South America and Australia (the fluvifaunulae delimited by Iredale & Whitley [15]).

Several zoogeographical and ecological categories can be recognized within these three groups, according to the age in fresh water and to salt tolerance. The three categories of fresh-water fishes proposed by Myers [18] [19] — primary, secondary and peripheral (with several subdivisions) — received almost general acceptance; his vicarious subdivision of peripheral fresh-water fishes must be considered an independent category [1]. These categories are based mainly on general salt-tolerance of families. Starobogatov [24] distinguishes three categories among fresh-water molluscs — paleo-, meso- and neolimnic — according to their age in fresh waters. These categories do not correspond to Myers' ones; e.g. fresh-water mussels, included by Starobogatov within mesolimnic animals because of the very old (Palaeozoic!) occurrence of some presumed ancestors or relatives in the sea, are, since late Mesozoic times, as salt-intolerant as primary fresh-water fishes. On the contrary,

the "paleolimnic" Viviparidae (with fossil record only in inland waters) have a certain degree of salt-tolerance, which enabled them to colonize, unlike primary fresh-water fishes, the Lesser Sunda Islands, New Guinea and Australia, starting from East Asia. Starobogatov ascribes to the mesolimnic division also some genera belonging to prevailing marine families, such as *Theodoxus*; adopting Myers' classification, these genera would be included within the vicarious division.

Starobogatov discusses also the status of fresh-water Crustacea, ascribing crayfishes, fresh-water crabs and Aeglidae to the mesolimnic, fresh-water prawns to the neolimnic division. Actually crayfishes are as intolerant to salt water as primary fresh-water fishes; the same is true for Aeglidae and for most families of fresh-water crabs, except Sundatelphusiadae which reached New Guinea and Australia [7] and Pseudotelphusidae which reached the West Indies [5] [6]. (Yet the families of fresh-water crabs have only a Tertiary age). Fresh-water Peracarida are rather recent intruders in fresh water, including, according to Henry & Magniez [11], the Asellidae, which were until recently considered an old fresh-water family. Peracarida belong thus partly to secondary, partly to peripheral, respectively to the meso- or to the neolimnic divisions.

b) **Primary aquatic animals inhabiting temporary pools and having possibilities of passive dispersal**

Many groups of small fresh-water invertebrates (Rotatoria, Cladocera, etc.) have large possibilities of passive dispersal, most or many of their species being cosmopolitan; these groups have no zoogeographical importance. Yet some groups of Entomostraca, although able to be transported (carried) by wind, etc. include mainly or exclusively species and genera with a restricted (in some cases even very restricted) range. This is the case especially with Anostraca, Conchostraca, and many Diaptomid Copepoda occurring only in temporary pools. The most important among them are the Anostraca.

This category of fresh-water animals includes much fewer species than the first mentioned and their distribution is incompletely known. Yet, according to the data so far available, the ranges of these species do not correspond to river drainages, but rather to climatic zones, resembling thus those of terrestrial animals. It seems even that the range of some species, e.g. *Limnadia lenticularis*, *Chirocephalus diaphanus*, *Ch. josephinae*, *Hemidiaptomus amblyodon*, corresponds to those of arboreal animals, the ranges of other species (*Branchinecta orientalis*, *Chirocephalus bobriskii*, *Arctodiaptomus salinus*) to those of eremial animals, while that of *Hemidiaptomus hungaricus* corresponds to the limit between arboreal and eremial areas. In North America, some species, such as *Branchinecta lindahli* live in arid areas both west and east of Rocky Mountains, unlike the animals of the first category, for which this mountain range marks a sharp zoogeographical boundary.

c) Fresh-water animals of terrestrial origin: insects and water mites

Practically all aquatic insects can fly, the flight possibilities being greater in Coleoptera, Hemiptera (both groups aquatic also as adults) and especially in Odonata, than in Plecoptera, Ephemeroptera, Trichoptera and some families of Diptera, such as Chironomidae, whose terrestrial adults live quite a short period and usually do not move from the vicinity of the water body from which they emerged; nevertheless, they can occasionally be carried on by the wind and are sometimes met in the aero-placton [9].

Most insects can thus cross water sheds; the same is true for most water mites, which live a period as parasites on water insects and can be carried on by these.

Very many genera of Coleoptera, Hemiptera and water mites have a quite wide range, many are even cosmopolitan. The zoogeography of these animals is little known, especially in tropical countries. The distribution of species seems to depend rather on climatic conditions (like that of terrestrial animals) than on river drainages.

The zoogeographically most interesting aquatic insects are the high rheophilic ones: most Plecoptera, many Ephemeroptera and Trichoptera. Many or most of these insects are confined to a certain mountain range, living on both slopes, e.g. in different drainages (Illies [14] a.o.). The same distribution pattern is shown by South American rheophilic water mites [3]. Plecoptera and other rheophilic water insects are abundant in hilly islands, such as Corsica, whose fauna of primary freshwater fishes and higher Crustacea is very poor or lacks even totally. This again proves the possibilities of these insects to cross narrow sea arms.

2. HYPOGEOUS FRESH-WATER ANIMALS

Some subterranean fresh-water animals are offshoots of recent or remote marine ancestors, having colonized underground inland waters (caves, wells, interstitial or hyporheic biotope) directly from the sea, while some others are derivatives of epigeous fresh-water animals. The intensive studies carried out in the last years on the subterranean aquatic fauna by tens of students in many countries have shown that many groups, until recently considered derivatives of surface fresh-water ancestors, actually are marine intruders (*Niphargus* and several other genera of Amphipoda [22]; the Cuban Brotulidae [8]), while other groups (e.g. the Asellidae [11]) are marine derivatives having colonized firstly surface, then subterranean fresh waters.

Many subterranean fresh-water animals of direct marine derivation have a quite restricted range, apparently indicating the restricted area in which their ancestors entered subterranean inland waters. Ruffo [22] for ex. lists 9 genera of hypogeous Amphipoda confined to the circummediterranean area and which originated from the Mediterranean sea. Several other genera extend rather far into Central Europe, yet their distribution suggests that they colonized continental subterranean waters

from the Oligocene [4] or Miocene [13] sea and since those periods did not extend their ranges. To such "conservative" hypogeous fresh-water animals belong the Ingolfiellidae, considered [23] to have a very old, probably Mesozoic, age in subterranean fresh waters and to have retained their initial colonization places, without reaching further inland. In many cases, each inland water species among these groups seems to have a distinct marine ancestor; few of them have speciated in fresh waters (e.g. the Ingolfiellid genus *Leloupiella* with one species in Congo and one in South-West Africa). In some cases a primary marine species colonized subterranean fresh water on a wide range: e.g. *Angeliera phraeaticola*, occurring in South Europe, India and Madagascar, which is surely a quite recent marine intruder. Also *Pseudoniphargus africanus* seems to have reached its range (North Africa, Madeira, South France, Dalmatia) by independent colonization from the sea, but has an older age in fresh water than the first species.

A few genera of marine descent have, on the contrary, undergone a rich speciation in subterranean fresh water and their species have dispersed by continental route. A typical case is that of *Niphargus*, an almost Pan-European genus (but absent from Spain, South Italy, etc.), which may have entered subterranean fresh waters from the Sarmatian Sea [22] or from a Miocene or Oligocene northern arm of the Tethys.

Many subterranean aquatic animals are offshoots of epigeous fresh-water ancestors, either primary fresh-water (Amblyopsidae and 19 species in 7 families of Ostariophysi among fishes, then some Rissoid snails, crayfishes, triclads, etc.), or more or less recent marine derivatives, having at first colonized surface fresh waters (practically all Asellota, some Amphipoda — e.g. *Crangonyx*, eventually the Syncarida), or even secondary aquatic groups of terrestrial origin (water mites). Most of them have a quite restricted range, indicating, as in the case of recent marine intruders, the area in which the colonization of subterranean water took place. Some hypogeous animals are ascribed to distinct genera because of the rather strong morphological differences between them and their epigeous relatives; yet the genetical differences prove in many cases to be slight (and thus the derivation recent): this is the case with *Anoptichthys* (actually not generically distinct from the surface genus *Astyanax*), and the same may be with *Typhlogarra*, *Coecobarbus*, etc.

Among the few exclusively hypogeous fresh-water animals with a wider range there are: some species of *Proasellus*, the genus *Crangonyx* (and some of its species), eventually some Bathynellacea (practically only *Bathynella natans*, *Parabathynella stygia*, *P. valdiviana* and *Leptobathynella riegellorum* according to Noodt [20]; actually the first named seems to be an artificial assemblage: Şerban [24]), comparatively few among the very numerous phraeatic and hyporheic water mites.

Our present knowledge about the distribution of the few subterranean fresh-water animals with a rather wide range, either of marine (e.g. some species of *Niphargus*) or of fresh-water origin (some *Proasellus*, *Crangonyx*, water mites, eventually a few Bathynellacea, etc.) and about subterranean hydrography are insufficient to permit a correlation between the ranges of species and present or past (surface and underground) hydrography or climatic zones.

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REPRODUCTION AND ONTOGENETIC DEVELOPMENT OF *PROTRACHEONISCUS POLITUS*-C CL KOCH

BY

NICOLAE TOMESCU

In this paper the author gives data concerning the reproduction and the postembryonal ontogenetic development of *Protracheoniscus politus*. He studied the period when the gestant females appear, the duration of gestation and the number of eggs laid by a female. He studied the development stages of the ontogenetic development and described the evolution of the morphological characters which have a taxonomic value. Based on his observations and on the bibliography, he found that various stages of the ontogenetic development in *Protracheoniscus politus*-Koch, beginning with the immature stage, were described as species and subspecies by different systematicians who did not know the ontogenetic development in this species.

The present paper presents data concerning the reproduction and the postembryonal development of the terrestrial isopode species *Protracheoniscus politus*, widely spread in the litter fauna of leafy forests of our country.

The researches on the reproduction and ontogenetic development of this species are very poor. B. Dominiak [3], trying to clear up the systematics of some species and sub-species of *Protracheoniscus*, gave data concerning the variability of secondary sexual characters based on the differences of size and age in the studied animals.

MATERIAL AND METHOD

We used animals collected from the ground at two weeks interval during the years 1968 and 1969. All the individuals were from the same population, thus belonging to the same species, *Protracheoniscus politus*, so that any taxonomic error is out of the

question. On the whole we collected 1,233 individuals, of which 220 males, 307 females and 706 larvae. For the study of larvae immediately after hatching out we brought gestant females which we kept in the laboratory until the hatching of larvae. We could maintain the larvae in laboratory conditions until the age of 2 months, when they died. We found that the animals belonging to this species do not tolerate well laboratory conditions. All the individuals were studied with the stereomicroscope, their body size was measured with ocular micrometer. From the males we made microscopical preparations with the appendix and parts of the body which have taxonomic value, beginning with the immature stage until the adult one. All the preparations were studied and drawn at the microscope in order to detect the evolution of ontogenetic characters and of the aspects of individual variability.

RESULTS

Reproduction. Our researches showed that the gestation period of *Protracheoniscus politus* lasts from the beginning of June till about the end of July, a period of 45—55 days. It may be shorter or longer, depending on the air temperature during the months when the females are gestant [1], [2], [7]. The females have a single laying a year (11—35 eggs); we very seldom found females in the third year of life who had two consecutive layings, the second one in the month of August. Only a very small number of the second laying larvae survive and reach sexual maturity at the same time with the first laying larvae, but remain much smaller in size. The death of a great number of larvae from the second laying is due to the fact that they are hatching out in August which in most cases is the most arid month of the year. It is probable that the *Protracheoniscus politus* larvae are insufficiently adapted to dryness conditions.

The *Protracheoniscus politus* females reach sexual maturity towards the end of the second year of life and the majority of them have a single laying, after which they die. Few adult females — about 23% — live for three years and have a second laying in the third year of life. If we compare this species with *Trachelipus balticus* [5] we find that the *Protracheoniscus politus* females have a much reduced prolificity. However, the individuals of *Protracheoniscus politus* have a relatively greater density in the litter fauna of leafy forests. This species is probably better adapted to the conditions of the biotope which they populate and has not so many enemies. In another paper in which we studied the dynamics of *Protracheoniscus politus* population we found that the survival coefficient of larvae is high, of about 5%.

Ontogenetic development. The description of postembryonary ontogenetic development will be made according to the same criteria used in another work [5] for *Trachelipus balticus*. We shall describe the larval period without subdividing it in stages (I, II, III, IV), since the morphological modifications occurring during this period are not significant from a systematic point of view.

At the moment of hatching the larvae are very poorly pigmented, the VIIth thoracic segment is incompletely developed, the lateral lobes of the head are absent (Fig. 1). The seventh pereiopode is missing and also the first pair of pleopodes. The body size of the animals is of 2—2.5 mm.

After about 20 days (in the second half of August), the seventh pereiopode appears, like a filament attached to the ventral part of the thorax. The VIIth thoracic segment is completely formed but is narrower than the precedent ones. In this stage the animals have a body size of 2.5—2.8 mm.
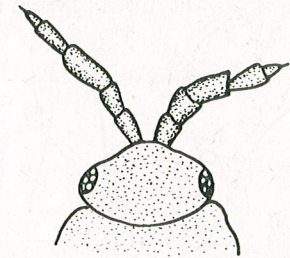

Fig. 1. — *Protracheoniscus politus* larva head immediately after hatching.

About 70—80 days after the hatching out (in October), the VIIth thoracic segment is completely developed and also the seventh pereiopode, which becomes functional. The larvae reach the length of 3.5—4.5 mm. Generally, the body shape, seen on the dorsal part, is similar to that of the adult, particularly the lateral lobes of the head (Fig. 2 *a*) and the pleotelson with the uropodes (Fig. 2 *b*). The larval period lasts for about 11—12 months. At its end the animals have a length of 4—4.5 mm. During this period the animals moult 5—6 times.
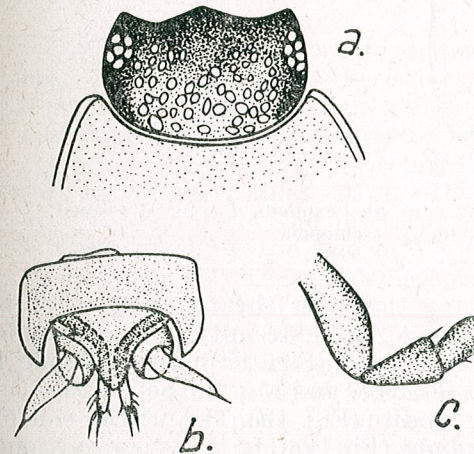

Fig. 2. — *Protracheoniscus politus* larva at the age of 8 months. *a* = head, *b* = pleotelson, *c* = pereiopode VII ischiopodite.

The immature stage appears at the first individuals of *Protracheoniscus politus* in about the second half of June, the following year, when the larvae are 11—12 months old. This stage begins with the modification of the pleopode I endopodite, which in males will become gonopode. At the beginning, the pleopode I endopodite has the form of an almost triangular blade (Fig. 3a) but it grows long, having the form of the adult gonopode (Fig. 3b), though without the terminal spur and the lateral lobe.

In the immature, the pleopode I exopodite is less similar to that of the adult (Fig. 3c). There are differences against the adult in the pleopode II exopodite and endopodite, too (Fig. 3d). The pereiopode VII ischiopodite has a straight inferior side (Fig. 3e). The immature stage lasts until about the end of July and the beginning of August. During this period the animals moult twice and reach a body size of 5—7 mm.
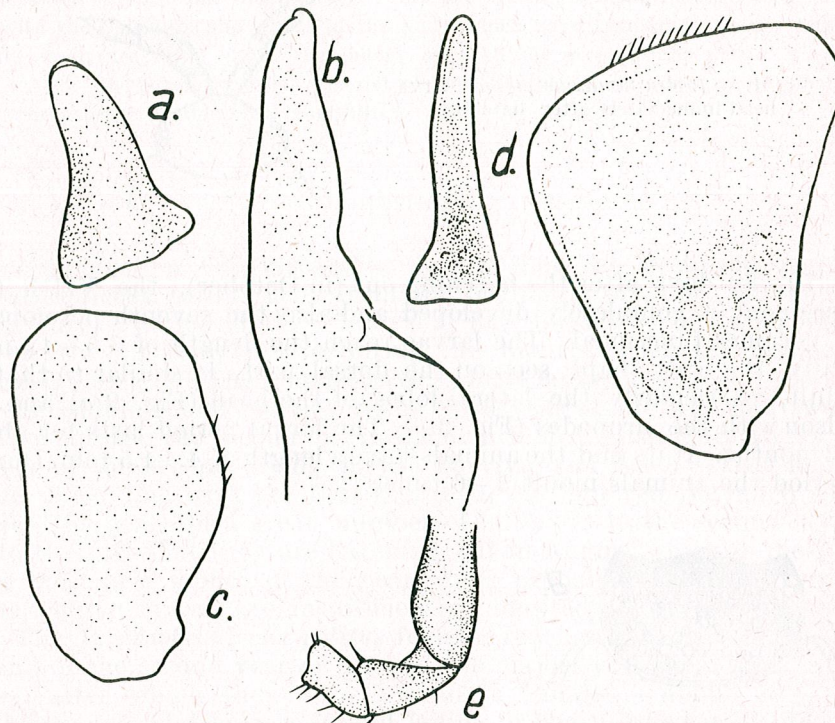

Fig. 3. — Immature stage. a, b, = pl. I end., c = pl. I exopod., d = pl. II exopod. and end., e = pereiopode VII ischiopod.

The juvenile stage appears 13 months after the hatching, that is in the month of August of the next year. At the extremity of the juvenile pleopode I endopodite a sharp spur is formed (Fig. 4 a), the pleopode I exopodite is similar to that of the adult (Fig. 4 b) and also the penis (Fig. 4 c) and the pleopode II exopodite and endopodite (Fig. 4 d). The inferior side of the pereiopode VII ischiopodite is straight (Fig. 4 e). In this stage only one moulting occurs and the juveniles have the body length of 5.5 — 7.5 mm.

The adult stage appears when the animals are 15 months old, that is in the second half of September. In this stage, at the extremity of pleopode I endopodite a chitinous lobe having small thorns and a subterminal position appears. The internal side of the terminal spur has a row of fine small teeth (Fig. 5 a). The pleopode I exopodite has the characteristic form of the species (Fig. 5 b). The pleopode II exopodite has a long stem which passes beyond the extremity of the exopodite (Fig. 5 c). The pleo-
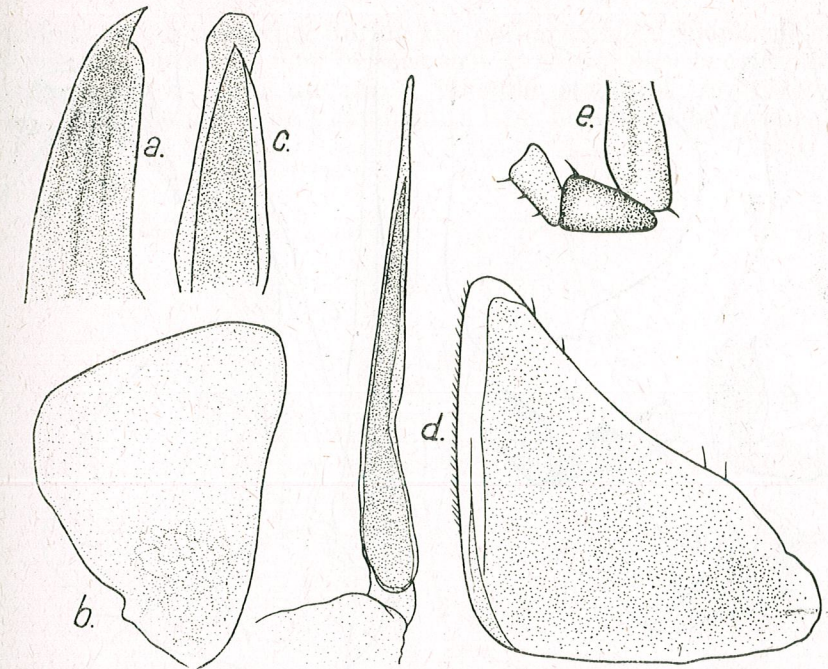
Fig. 4. — Juvenile stage. a = pl. I end., b = pl. I exopod., c = penis, d = pl. II exopod. and end., e = pereiopode VII ischiopod.

telson has the characteristic shape of the species (Fig. 5 d). At the beginning of the adult stage the ischiopodite of pereiopode VII has a straight inferior side (Fig. 5 e), then, a month after the appearance of the pleopode I endopodite lobe, a concavity on the inferior side of the ischiopodite appears (Fig. 5 f), which becomes deeper as the animal gets older (Fig. 5 g). In October, about 16 months after the hatching, the secondary sexual characters of the males of Protracheoniscus politus are definitively formed, reaching the size of 7—9 mm. The adult males will fecundate the females only the following spring, in April-May, that is at about the age of 1 year and 10 months. Approximately 2 months after the fecundation, in July, the adult males moult (the unique moulting of that year and the last of their life) and subsequently a part of the secondary sexual characters are modified, becoming similar to those of the juveniles. The lobe of the pleopode I endopodite disappears (Fig. 6 a), the stem of the pleopode I endopodite (Fig. 6 b) is reduced and the concavity of the pereiopode VII ischiopodite is attenuated (Fig. 6 c). These modifications last until the end of August and the beginning of September; afterwards the characters reappear, arriving again at the typical adult form. Meanwhile, many males die and only a very small number of them remain alive; but during wintertime they die, too. In May we did not find any more males of Protracheoniscus politus belonging to the 2—3 years age-class. It seems that the males of this species take part to a single fecundation and then die.
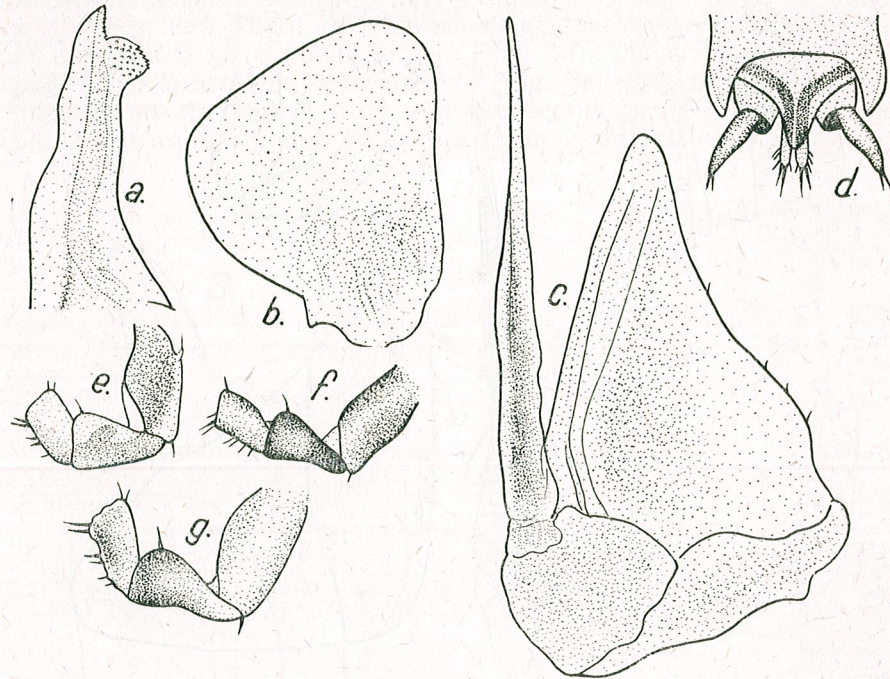
Fig. 5. — Adult stage. *a* = pl. I end., *b* = pl. I exopod., *c* = pl. II exopod. and end.,
d = pleotelson, *e*, *f*, *g* = pereiopode VII ischiopod.

If we analyze the appearance order of the secondary sexual cha-
racters we find that it is the pleopode I endopodite and exopodite which
are formed at first, the concavity of the pereiopode VII ischiopodite
appearing later.

Concerning the individual variation of the characters in males, we
found that in the pleopode I endopodite and the pereiopode VII ischio-
podite there are small, insignificant variations. On the other hand, we
found great variations of the shape and size of the pleopode I exopodite
(Fig. 7). Likewise there are important variations connected with the pig-
mentation and the body size of the animals. The variation of the body
size increases as the animals get older, so that it is difficult to determine
their age according to the body size only, particularly in autumn when
the juveniles appear. It is necessary to study the secondary sexual cha-
racters, too. It is to be noted that, generally, the females are smaller than
the males.

The study of the ontogenetic evolution of the characters shows
that in this species there are great variations determined by the age and
by the life cycle of the animals; therefore many authors considered them
stable characters belonging to different species [6], [7], [8]. We appreciate
that the present paper gives sufficient proofs supporting the observa-
tions and the proposals made by Dominiak [3] to synonymize a number
of species and subspecies of the genus *Protracheoniscus* which actually

are various stages occurring during the ontogenetic development of *Pro-
tracheoniscus politus*. It may be seen that *Protracheoniscus amoenas*-Koch
corresponds to a more advanced juvenile stage of *Protracheoniscus
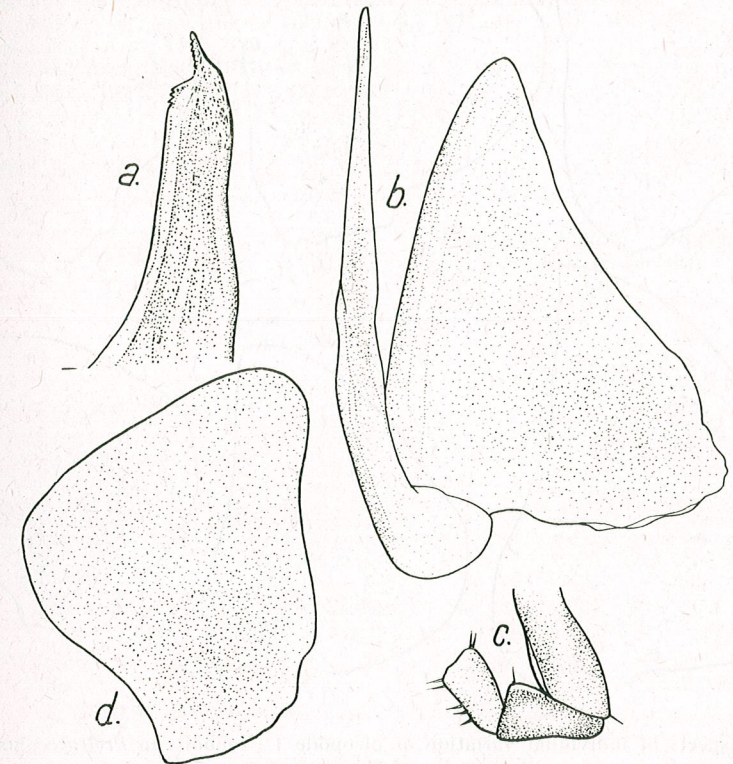politus*, when the pleopode I endopodite lobe begins to be formed. *Pro-*

Fig. 6. — Adult after moulting in July, *a* = pl. I end., *b* = pl. II exopod.
and end., *c* = pereiopode VII ischiopod., *d* = pl. I exopod.

tracheoniscus marcomanius-Verh corresponds to the immature stage and
Protracheoniscus politus slovakius-Strouh has the characters of the
Protracheoniscus politus adult after the last moulting. *Protracheoniscus
politus carpathicus*-Verh cannot be considered a genuine species only on
the basis of the different shape of the pleiopode I exopodite. As it is seen
in figure 7, this character is very unstable in *Protracheoniscus politus*.

CONCLUSIONS

In *Protracheoniscus politus* the incubation period lasts from the
beginning of June till the end of July, a period of 45—55 days. The fe-
males have a unique egg-laying (11—35 eggs), after which most of them
die. About 23% of the females live for 3 years and in the third year they
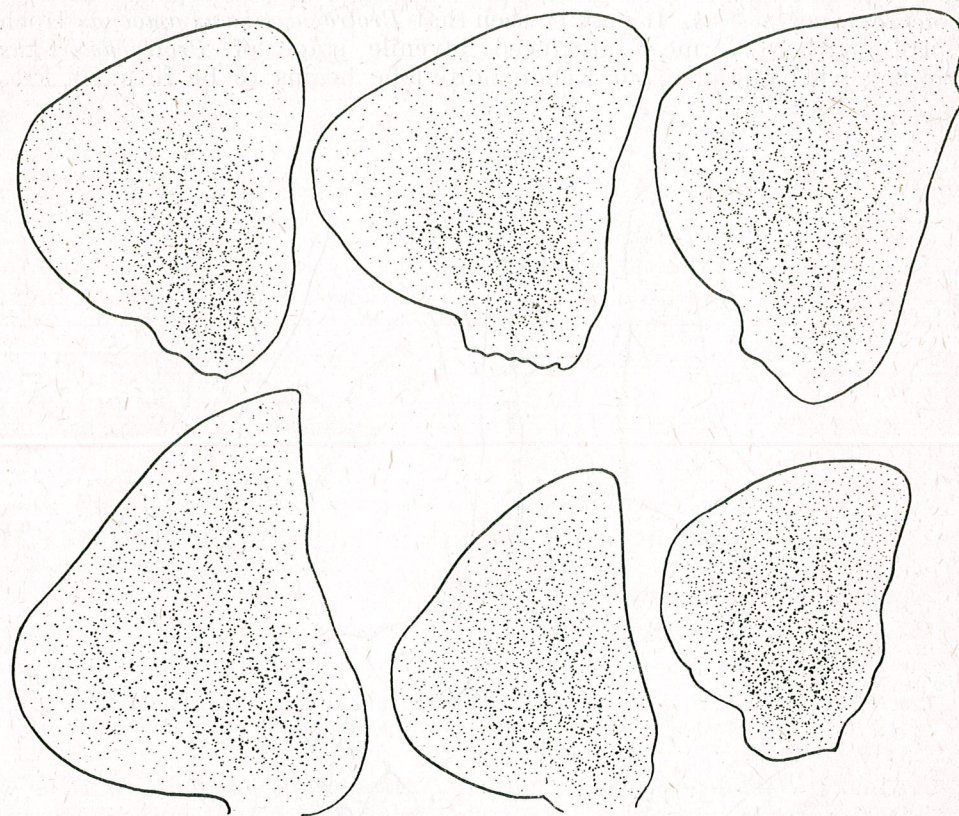have also one laying. Exceptionally, some 3-year-old females have two

Fig. 7. — Aspects of individual variation of pleopode I exopodite in *Protracheoniscus politus* adult males of the same age.

consecutive layings in the third year but the larvae resulting from the second laying die in a very great number.

The postembryonal ontogenetic development until the adult stage lasts about 15—16 months.

The adult males moult only once, after the females fecundation. After the moulting they change some of their secondary sexual characters, resembling the juveniles.

The individual variations are mainly determined by the age and the life cycle of the animals. In the males of the same age these variations are smaller.

The species : *Protracheoniscus amoenas*-Koch and *Protracheoniscus marcomanius*-Verh, and the subspecies : *Protracheoniscus politus slovakius*-Strouh and *Protracheoniscus politus carpathicus*-Verh correspond morphologically to some stage characters or individual variations of the species *Protracheoniscus politus*-Koch. In conclusion, we propose their synonymization with this species.

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FURTHER STUDIES ON THE EFFECT OF MONOFLUOR-ACETATE ON THE GLUCONEOGENESIS

BY

GH. FRECUŞ, E.A. PORA and I.V. DEACIUC

Effects of monofluoracetate (MFA) on the glucose synthesis from various precursors in bovine and rat kidney cortex slices and in rat liver slices were studied. In bovine kidney cortex slices MFA produced a marked decrease of glucose synthesis with lactate as substrate, but did not affect it when precursors of glucose were 2-oxoglutarate, malate or succinate. Simultaneously, MFA depressed oxygen consumption with lactate and did not affect it with other substrates. In rat kidney cortex slices, MFA depressed oxygen consumption and glucose synthesis with succinate as substrate, but not with 2-oxoglutarate. Finally, in rat liver slices MFA inhibited glucose synthesis with lactate, pyruvate and glycerol 1-phosphate. The results are interpreted as suggesting that hyperglycemia induced *in vivo* by MFA cannot be attributed to an enhancement of gluconeogenesis, as postulated by Gulyi et al.

In a preceding paper [1] we have shown that monofluoracetate (MFA) inhibits glucose synthesis from a variety of precursors in rat kidney cortex slices. Since this effect of MFA was associated with a consistent depression of the rate of oxygen consumption, we supposed that one of the possible mechanisms of action of this inhibitor on gluconeogenesis consists in a depression of ATP generation.

The present paper presents the results obtained in experiments on bovine and rat kidney cortex slices, showing that MFA, when it does not affect the oxygen consumption by the slices, has no effect on the ability of slices to synthesize glucose.

In addition, in this paper data are presented showing that MFA inhibits gluconeogenesis in rat liver slices. These data, corroborated with those of a preceding paper [1], make no longer valid the hypothesis of

Gulyi et al. [4], according to which the hyperglycemia induced by MFA injected to rabbits and rats [2] [4], is due to an enhancement of gluconeogenesis by this poison.

MATERIALS AND METHODS

Chemicals. Peroxidase, type II from horse-radish, was purchased from Mann Research Lab. Inc., U.S.A., while glucose oxidase was kindly supplied to us by Prof. M.F. Gulyi, from the Institute of Biochemistry, Kiev, U.S.S.R. All other chemicals were of the highest purity commercially available.

Animals. Bovine kidneys from donors of about two years old were obtained from the local slaughter-house and rapidly transported into laboratory, on ice. The time elapsing between the killing of animals and the moment when the tissue slices were placed in incubation vessels was of 45—60 min. Rat livers were obtained from white male rats, weighing 180—230 g., maintained on a Larsen diet, and fasted 24 hours prior to experiment, with water *ad libitum*. The rats were killed by a blow on the head, followed by decapitation and exsanguination.

Incubation procedure and assay of glucose. The procedure described below refers only to the experiments performed on bovine kidney cortex slices and on rat liver slices. Experiments on rat kidney cortex slices were performed exactly as described elsewhere [1].

Slices of both bovine kidney cortex and rat liver were made free hand with the aid of a razor blade. The incubation media were the saline of Krebs et al. [7] for the slices prepared from bovine kidney cortex, and the Krebs-Ringer bicarbonate buffer [10] for the slices prepared from rat liver. After being washed in the corresponding cooled salines, the slices were blotted on a filter paper, rapidly weighed on a torsion balance and placed in 20 ml. Warburg vessels, containing the incubation medium and additions as indicated in the tables. All the substrates and the inhibitor were dissolved in the medium used for incubation and, when necessary, neutralized to pH 7.4 prior to addition to the vessels. Oxygen consumption was measured only for kidney cortex slices and, for this purpose, 0.40 ml. of 15 % (w/v) KOH was placed in the centre well of the vessels. The gaseous phase was oxygen (100 %) in experiments with kidney cortex slices, and oxygen : CO_2 (95 : 5 %) in those with liver slices. Incubation was carried out for 1 hour, at 39° in a final volume of 4.0 ml. for kidney cortex slices, and for 2 hours, at 37.5° in a final volume of 3.0 ml. for rat liver slices. In both cases the shaking velocity was of 140 strokes per min.

At the end of incubation of bovine kidney cortex slices, these were rapidly removed and 1.0 ml. of 1.5 N $HClO_4$ was added to the remainder in the vessels. After centrifugation at 0—4°, an aliquot (usually 1.0 ml.) of clear supernatant was used without prior neutralization for glucose assay (see below).

In experiments with rat liver slices, at the end of incubation 0.5 ml. of 4.2 N H_2SO_4 was added to the content of the vessels (i.e. the medium plus the slices) and the mixture was refluxed for 2.5 hours on a boiling water bath. Then, the mixture was made up to 25 ml. and, after centrifugation, 1.0 ml. was taken for glucose assay, without prior neutralization. In this procedure the glycogen initially present in the slices as well as that synthesized during incubation is hydrolysed to glucose. In this way the net carbohydrate synthesis in the slices can be easily measured without separate measurement of glucose liberated into medium and of glycogen content of the slices.

Glucose assay was conducted essentially as described by Krebs et al. [5], except that at the end of incubation of 1.0 ml. of unknown solution (see above) with 3.0 ml. of glucose oxidase reagent, 4.0 ml. of 50 % (v/v) H_2SO_4 were added under cooling [9], and the optical

Table 1

Effect of monofluoroacetate (MFA) on glucose synthesis and oxygen consumption by bovine kidney cortex slices in the presence of various precursors

Precursor and concentration	MFA (5 mM)	Glucose formed (μmoles/g. tissue dry weight/hour)*	Inhibition due to MFA (%)	Oxygen consumption (μmoles/g. tissue dry weight/hour)*	Inhibition due to MFA (%)
No substrate	−	8.9 ± 0.3 (11)		371 ± 10 (9)	
	+	6.1 ± 0.5 ** (9)	33.8	255 ± 15 ** (8)	47.8
L-Lactate	−	16.8 ± 0.5 (9)		408 ± 15 (7)	
(10 mM)	+	8.8 ± 0.8 ** (8)	47.7	303 ± 20 ** (6)	25.8
2-Oxoglutarate	−	18.6 ± 1.7 (7)		480 ± 18 (5)	
(10 mM)	+	21.3 ± 2.1 (7)	−	492 ± 22 (5)	−
Succinate	−	36.1 ± 5.4 (5)		674 ± 42 (4)	
(10 mM)	+	37.7 ± 2.5 (5)	−	702 ± 12 (4)	−
DL-Malate	−	25.5 ± 2.3 (8)		457 ± 19 (6)	
(20 mM)	+	26.5 ± 2.8 (7)	−	409 ± 23 (5)	−

Tissue slices, corresponding to 9—13 mg. of dry weight, were incubated as stated in the Materials and Methods section. * Values in the column represent mean ± standard error of the mean, with the number of animals in parentheses. ** The difference from the appropriate control, i.e. slices incubated without inhibitor, is statistically significant at P < 0.01.

Table 2

Effect of monofluoracetate (MFA) on glucose synthesis and oxygen consumption in rat kidney cortex slices in the presence of succinate and 2-oxoglutarate

Precursor and concentration	MFA (5 mM)	Glucose formed (μmoles/g. tissue dry weight/hour)*	Inhibition due to MFA (%)	Oxygen consumption (μmoles/g. tissue dry weight/hour)*	Inhibition due to MFA (%)
No substrate	−	13.8 ± 1.3		502 ± 25	
	+	3.1 ± 0.4 **	77.6	247 ± 21 **	60.8
Succinate (10 mM)	−	86.6 ± 5.2		795 ± 42	
	+	38.4 ± 4.4 **	55.7	607 ± 36 **	23.7
2-Oxoglutarate (10 mM)	−	89.1 ± 6.3		576 ± 36	
	+	90.6 ± 5.4	—	582 ± 22	—

Tissue slices, corresponding to 6—8 mg. dry weight, were incubated in a final volume of 2.0 ml. for 1 hour, exactly as previously described [1]. * Values in the column represent mean ± standard error of the mean obtained on 8 animals. ** The difference from the appropriate control, i.e. slices incubated without inhibitor, is statistically significant at P < 0.01.

density was measured at 545 mμ in a Zeiss VSU−1 spectrophotometer. The final acidity of both solutes to be assayed for glucose was too low to make necessary their neutralization prior to mixing with the glucose oxidase reagent. For each set of analyses at least two glucose standards were parallelly performed.

The statistical treatment of the data was done according to the Student t test. P values of 0.05 or less are considered to be statistically significant.

Other experimental details are given in the legends to the tables.

RESULTS

The data presented in table 1 show that in bovine-kidney cortex slices MFA inhibited glucose synthesis from lactate and did not affect it when 2-oxoglutarate, succinate or malate served as glucose precursors. On the other hand, MFA depressed oxygen consumption only with lactate as substrate and had no effect on this parameter when respiratory substrates were 2-oxoglutarate, succinate or malate. In other words, the inhibitory effect of MFA on the gluconeogenesis is constantly associated with its depressing action on the oxygen consumption.

A similar relation between the effect of MFA on gluconeogenesis and its effect on oxygen consumption is recorded for rat kidney cortex slices (Table 2). Thus, MFA strongly diminished glucose synthesis from succinate and, simultaneously, lowered the oxygen consumption by the slices in the presence of this substrate. Neither oxygen consumption, nor glucose synthesis were affected by MFA when 2-oxoglutarate served as substrate.

Finally, the data presented in table 3 clearly show that MFA exerted a strong inhibitory effect on the gluconeogenesis from lactate, pyruvate and glycerol 1-phosphate, in rat liver slices.

Table 3

Effect of monofluoracetate (MFA) on glucose synthesis from various precursors in rat liver slices

Precursor and concentration	MFA (5 mM)	Glucose formed (μmoles/g. tissue dry weight /2 hours)*		Inhibition due to MFA (%)
No precursor	−	42.4 ± 2.6	(7)	
	+	13.9 ± 3.4 **	(7)	67.3
L-Lactate (20 mM)	−	171.5 ± 10.6	(8)	
	+	93.0 ± 8.9 **	(8)	45.8
Pyruvate (20 mM)	−	93.3 ± 4.4	(5)	
	+	45.0 ± 10.8 **	(5)	51.8
DL-Glycerol 1-phosphate (20 mM)	−	95.8 ± 7.0	(5)	
	+	47.8 ± 4.6 **	(5)	51.2

Tissue slices, corresponding to 11—14 mg. of dry weight, were incubated as stated in the Materials and Methods section. * Values in the column represent mean ± standard error of the mean, with the number of animals in parentheses. ** The difference from the appropriate control, i.e. slices incubated without inhibitor, is statistically significant at P < 0.01. The initial content of carbohydrate in the slices averaged 15.4 ± 2.8 (n = 8); this value was subtracted from the total carbohydrates found in the medium plus the slices at the end of the incubation.

DISCUSSION

Our results obtained on bovine kidney cortex slices show that MFA exerts an inhibitory effect on both glucose synthesis and on oxygen consumption in the presence of lactate. The same was observed in experiments on rat kidney cortex slices [1]. We supposed that this effect of MFA is due to an impairment of ATP generation, among other factors previously discussed [1].

The interesting point of the data presented in this paper consists in the fact that glucose synthesis in kidney cortex slices from various precursors was inhibited by MFA only when the oxygen consumption, with the same precursors, was lowered by the poison. This is the case with 2-oxoglutarate, malate and succinate in bovine kidney cortex slices, and with 2-oxoglutarate in rat kidney cortex slices. Thus, it appears that the effect of MFA on gluconeogenesis depends upon its action on the oxygen consumption. This gives a further support to our previous assumption [1], according to which one of the possible mechanisms by which MFA inhibits gluconeogenesis is the decrease of ATP to ADP ratio and, as a consequence, the diminution of flux through ATP-dependent steps of gluconeogenesis.

When the glucose synthesis is proceeding from lactate, the tissue must oxidize a portion of lactate in order to ensure the conversion into glucose of the remainder lactate [6]. In the presence of MFA the oxidation of lactate (through pyruvate to CO_2) is impaired due to the blockage of citric acid cycle at the aconitase step [8]. As a consequence, the generation of ATP is not sufficient to sustain a consistent glucose synthesis from lactate. When, however, the precursors of glucose are dicarboxylic acids, like 2-oxoglutarate, succinate or malate, the ATP generation may be sufficient to support a gluconeogenesis, even when citric acid cycle is blocked at the aconitase step. This accounts for the results presented in this paper, obtained on bovine kidney cortex slices with 2-oxoglutarate, succinate and malate, and for those obtained on rat kidney cortex slices with 2-oxoglutarate. This assumption cannot, however, be applied to explain the inhibitory effect of MFA on glucose synthesis by rat kidney cortex slices with malate [1] or succinate (Table 2) as precursors.

It appears that in cortex slices of rat kidney, but not of bovine kidney, fluorocitrate, formed from monofluoracetil-CoA and oxaloacetate, is an inhibitor not only of aconitase, but also of other enzymes. It has been shown that fluorocitrate is a potent inhibitor of succinate dehydrogenase from rat kidney [3]. This can account for the depression by MFA of oxygen consumption with succinate in rat kidney cortex slices (Table 2). The fact that such an effect of MFA was not observed in bovine kidney cortex slices, incubated with succinate, may be due to a low ability of this tissue to synthesize fluorocitrate, or to a lack of an effect of this compound on succinate dehydrogenase.

The mechanism by which MFA inhibits oxygen consumption by rat kidney cortex slices, in the presence of malate, remains unknown.

Finally, our results show that MFA is an inhibitor of gluconeogenesis not only in kidney cortex, but also in liver. This is an important point for the understanding of the nature of hyperglycemia observed *in vivo* in MFA-poisoned animals [2] [4], since beside the kidney cortex, liver is another major site of gluconeogenesis in mammals. In this way, it becomes evident that MFA-induced hyperglycemia cannot be attributed to an enhancement of gluconeogenesis in either kidney cortex or liver.

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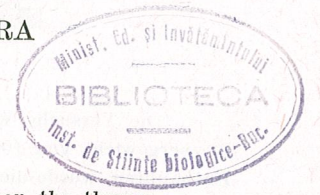
DER EINFLUSS DES MADIOLS * AUF DIE THYMUS-ATROPHIERENDE WIRKUNG DES HYDROCORTISONS UND AUF DEN GLYKOGENGEHALT DES THYMUS UND DER LEBER BEI WEISSEN RATTEN

J. MADAR, V. TOMA und E.A. PORA

The effect of Madiol (17α-methyl-androst-4-en, 3β, 17β-diol) upon the thymolytic activity of Hydrocortisone, as well as upon the glicogen content of the thymus and liver in white rats was followed.

It was established that Madiol after a 3-days treatment with a daily dose of 5 mg/100 g induced a marked thymus involution, the metabolic mechanism of which differed from that of the involution induced by Hydrocortisone.

In the case of an association of Hydrocortisone with Madiol in equal doses or in a ratio with quantitative preponderance of Madiol, the latter did not intensify the thymolytic activity of Hydrocortisone, but reduced the glycogen loading of thymus induced by Hydrocortisone alone.

In den letzten Jahren wurden Glucocorticoide in immer größerem Ausmaß in der Therapie und in der Kinderheilkunde verwendet. Neben ihrer spezifischen Heilwirkung lösen die Glucocorticoide eine überaus heftige Involution des Thymus aus [2], [13], was für die Ausübung des immunologischen Schutzes im jugendlichen Organismus äußerst negative Folgen haben kann [3], [16].

In der Klinik finden neben den Glucocorticoiden eine Reihe anaboler Steroide, die die Eiweißsynthese in den Geweben sowie das gesamte Wachstum des jugendlichen Organismus anzuregen vermögen, breite Verwendungsmöglichkeiten [4]. Die antikatabole Wirkung der

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* Das Madiol (17α-Methyl-androst-4-en-3β,17β-diol) wurde bei BIOFARM — Bukarest hergestellt.

anabolen Steroide, die durch eine Verringerung des Glucocorticoid-
bedingten Eiweißabbaus zustandekommt, konnte in wiederholten Unter-
suchungen nachgewiesen werden [1], [4], [5], [15]. Die Herabsetzung
der Glucocorticoiddosen ist bei gleichbleibender pharmakologischer
Wirkung, auf diese Weise durchaus möglich, wenn die Glucocorticoid-
behandlung mit der Verabreichung anaboler Steroide kombiniert wird
[1], [15].

Von dem Gegensatz in der Wirkungsweise der Glucocorticoide und
der anabolen Steroide ausgehend, untersuchten wir den Einfluß des
Madiols auf die dem Hydrocortison eigene Fähigkeit der Thymusinvo-
lution. Da zwischen dem Thymusinvolutionsvermögen und der glyko-
genetischen Wirkung der Glucocorticoide Wechselbeziehungen bestehen
[14], und da im Falle der vom Hydrocortison hervorgerufenen Involu-
tion des Thymus ein gleichzeitiges Zunehmen seiner Glykogenreserven
nachgewiesen werden konnte [11], verfolgten wir in den vorliegenden
Untersuchungen den Glykogengehalt des Thymus und der Leber.

MATERIAL UND METHODEN

Die Versuche wurden mit weißen Wistar-Ratten, weiblichen Geschlechts und einem
Körpergewicht von 100—120 g durchgeführt. 18 Stunden vor dem Opfern der Tiere, durch
Enthaupten, wurde diesen das Futter entzogen.

Wir führten folgende Versuchsvarianten aus:

Kontrollgruppe I — mit physiologischer Kochsalzlösung injiziert;

Gruppe II — 3 Tage lang mit Hydrocortison („BIOFARM"), in täglichen Dosen von
5 mg/100 g behandelt;

Gruppe III — 3 Tage lang mit Madiol, in täglichen Dosen von 5 mg pro 100 g behan-
delt;

Gruppe IV — 3 Tage lang mit 5 mg Madiol + 5 mg Hydrocortison pro Tag pro 100 g
behandelt;

Gruppe V — 3 Tage lang mit 2,5 mg Hydrocortison/Tag behandelt und

Gruppe VI — 3 Tage mit 2,5 mg Hydrocortison + 5 mg Madiol/Tag behandelt.

Das Hydrocortison und das Madiol wurden intramuskulär verabreicht und die Tiere
wurden 24 Stunden nach der letzten Hormongabe getötet.

Der Thymus und Teile des Lebergewebes wurden nach dem Enthaupten der Tiere, so-
fort entnommen und auf einer Spiralfederwaage gewogen. Die Glykogenbestimmung erfolgte
nach der Methode von Montgomery [10].

ERGEBNISSE UND DISKUSSION

Die in Tabelle 1 und in Abbildung 1 enthaltenen Angaben des
Thymusgewichtes bestätigen die von der verabreichten Dosis abhängige
thymusrückbildende Wirkung des Hydrocortisons [13]. Das Thymus-
gewicht, mit 5 mg Hydrocortison pro Tag behandelter Tiere, fällt um 61,2%
den Kontrollen gegenüber ($p < 0,001$), während eine Gabe von 2,5 mg
pro Tag das Gewicht des Thymus nur um 46,6% ($p < 0,001$) sinken
läßt. Die Involution des Thymus wird von einer Zunahme des Glykogen-

gehaltes um 23% ($p < 0,05$) bzw. 32,7% ($p < 0,001$) begleitet. In
der Leber wurde eine signifikante Glykogenzunahme (66,5%, $p < 0,01$),
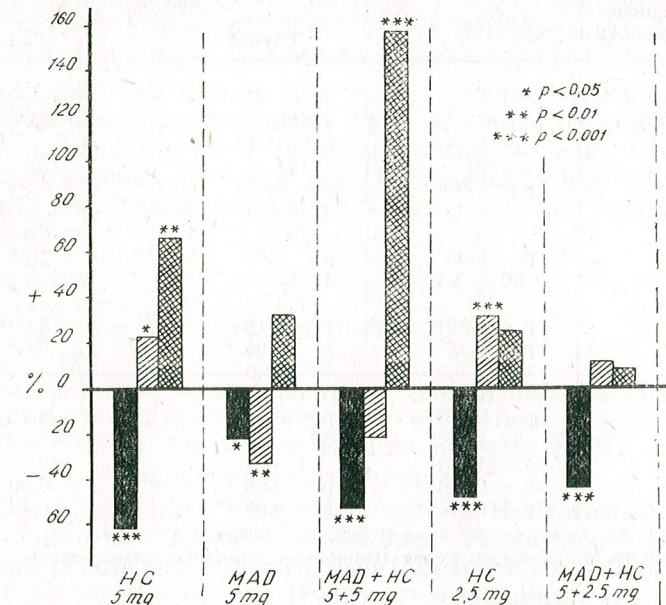nur bei einer Hydrocortisondosis von 5 mg pro Tag erzielt.

Abb. 1. — Prozentuelle Veränderungen des Thymusgewichtes,
sowie des Glykogengehaltes des Thymus und der Leber bei
weißen Ratten, als Folge einer 3 tägigen Behandlung mit
Hydrocortison (HC), Madiol (MAD), bzw. Madiol+Hydrocortison
(MAD+HC). Die Steroide wurden in täglichen Gaben von je 2,5
bzw. 5 mg/100 g i.m. verabreicht. Die erhaltenen Ergebnisse
wurden mit den Werten der Kontrollgruppe verglichen.

▉ = Thymusgewicht; ▨ = Glykogengehalt des Thymus; ▩ = Glykogengehalt
der Leber.

Die Madiolverabreichung vermindert das Gewicht des Thymus
ebenfalls wesentlich (21,8%, $p < 0,05$), aber in geringerem Ausmaß als
gleiche Gaben von Hydrocortison. Der Glykogengehalt des Thymus er-
fährt hingegen ein den durch Hydrocortison bedingten Veränderungen
entgegengesetztes, signifikantes Sinken den Kontrollen gegenüber (32,7%
$p < 0,02$), während der Glykogengehalt der Leber eine leicht steigende
Tendenz aufweist.

Die gleichzeitige tägliche Verabreichung von 5 mg Hydrocortison
und 5 mg Madiol verstärkt trotz eigener thymusrückbildender Wirkung
des anabolen Steroids, das Thymusinvolutionsvermögen des Hydro-
cortisons nicht ($p > 0,10$). Das Madiol gleicht hingegen die glykogene-
tische Wirkung des Hydrocortisons auf den Thymus aus, und senkt
den Glykogengehalt dieses Organs unter die Werte der Kontrollen. Gleich-
zeitig erhöht das Madiol den Leberglykogengehalt wesentlich ($p <
0,001$), durch eine Förderung der Hydrocortison-bedingten Gluco-
neogenese.

Tabelle 1

Thymusgewicht und Glykogengehalt von Thymus und Leber bei 3 Tage, mit täglichen Dosen von 5 oder 2,5 mg/100 g Hydrocortison, Madiol, bzw.Madiol + Hydrocortison behandelten weißen Ratten

Gruppe und tägliche Steroiddosis(mg/100 g)	Thymus mg	Glykogengehalt (mg%)	
		Thymus	Leber
I Kontrolle (Kochsalzlösung)	193 ± 8,8 (8)	52 ± 3,4 (8)	2765 ± 364 (8)
II Hydrocortison 5 mg	74 ± 4,5 (8) $p < 0,001$	64 ± 4,8 (8) $p < 0,05$	4605 ± 301 (8) $p < 0,01$
III Madiol 5 mg	151 ± 15,6 (8) $p < 0,05$	35 ± 3,2 (8) $p < 0,02$	3615 ± 377 (8) $p > 0,25$
IV Madiol + Hydroc. 5 + 5 mg	90 ± 9,4 (7) $p < 0,001$ $p > 0,10$ *	41 ± 6,8 (7) $p > 0,10$ $p < 0,05$ *	7133 ± 650 (8) $p < 0,001$ $p < 0,01$ *
V Hydrocortison 2,5 mg	103 ± 8,2 (9) $p < 0,001$	70 ± 2,6 (9) $p < 0,001$	3468 ± 262 (8) $p > 0,10$
VI Madiol + Hydroc. 5 + 2,5 mg	92 ± 7,4 (10) $p < 0,001$ $p > 0,25$ **	58 ± 4,7 (10) $p < 0,50$ $p < 0,05$ **	3024 ± 191 (9) $p > 0,10$ $p > 0,10$ **

* Verglichen mit den Werten der mit 5 mg Hydrocortison behandelten Gruppe.
** Verglichen mit den Werten der mit 2,5 mg Hydrocortison behandelten Versuchsgruppe.

Das Madiol hält seine eben beschriebene Wirkung auf den Thymus bei, auch wenn das Hydrocortison in verringerten Gaben von 2,5 mg pro Tag verabreicht wird. Der Glykogengehalt der Leber wird in diesem Falle jedoch nicht beeinflußt.

In vorangehenden Untersuchungen [11] gelang es uns nachzuweisen, daß die involutive und glykoneogenetische Wirkung des Hydrocortisons durch gleichzeitige, geringe Gaben von Insulin beeinflußbar ist. Dieser Umstand wurde durch eine Hemmung, des vom Hydrocortison ausgelösten Eiweißabbaus im Thymus, sowie durch die Hemmung der örtlichen Gluconeogenesevorgänge, die sich der aus den Thymuseiweißen abgespaltenen glukosebildenden Aminosäuren bedienen, erklärt [11]. In der Literatur wird verschiedentlich darauf hingewiesen, daß anabole Steroide einerseits die Eiweißsynthese fördern, anderseits aber auch die eiweißabbauende Wirkung der Glucocorticoide herabsetzen [1], [4], [5], [15]. Es gelang uns bereits nachzuweisen, daß bei weißen Ratten die Insulinsekretion und die Glukosetoleranz, durch Hydrocortison vermindert wird [7], [9], während das Madiol die insulinische Aktivität des Plasmas und die Glukosetoleranz steigert [8], [12]. Es war daher anzunehmen, daß das Madiol den Thymus vor dem involutiven Eingriff des Hydrocortisons gewissermaßen schützt. Trotzdem lassen unsere Ergebnisse auf das Vorhandensein verschiedener Wirkungsmechanismen des Insulins bzw. Madiols auf den Thymus schließen. Die vorliegenden Ergebnisse zeigen im Vergleich zur Wirkung auf den Thymus gänzlich andere Wirkungen im Falle der Leber.

Es konnte aber auf alle Fälle sichergestellt werden, daß das Madiol, obwohl zu den Steroiden mit allgemein anabolen Eigenschaften gehörend, ein ausgeprägtes Thymusinvolutionsvermögen aufweist, ein Umstand, der in der Kinderheilkunde in Betracht gezogen werden muß, da dem Thymus im Gefüge der immunologischen Abwehrreaktionen der Nachgeburtsperiode eine führende Aufgabe zufällt [3], [16].

Der Einfluß des Madiols dürfte durch den Umstand erklärbar sein, daß die während der Involution des Thymus freigesetzen glukosebildenden Aminosäuren nicht mehr an Ort und Stelle zu Glykogen umgebaut werden können, und daß der thymusrückbildende Effekt des Madiols andere Stoffwechselvorgänge auslöst als die Verabreichung von Glucocorticoiden.

SCHLUSSFOLGERUNGEN

1. Abschließend kann behauptet werden, daß das Madiol, ein anaboles Steroid, bei jungen Ratten eine wesentliche Involution des Thymus bedingt, wobei der ihr zu Grunde liegende Stoffwechselmechanismus von dem, des Hydrocortisons abweicht.

2. Die kombinierte Verabreichung von Hydrocortison und Madiol in äquivalenten Dosen, oder mit einem verringerten Hydrocortisonanteil, verstärkt die vom Hydrocortison induzierte Thymusinvolution nicht, verringert jedoch die für die Hydrocortison-bedingte Involution charakteristische Glykogenbereicherung des Thymus.

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THE EFFECT OF THIOUREA AND THYROID EXTRACT ON THE OXYGEN CONSUMPTION IN MOLLUSCS UNDER HYPOTHERMIC CONDITIONS*

BY

C. A. PICOŞ

The variation of oxygen consumption in molluscs (*Unio pictorum*) previously acclimatized under conditions of cold (5°—6°C) was investigated by giving them thiourea (1 g./l.) and thyroid extract (0.125 g./l.) in water.

The experimental results led to the following conclusions :

1. At 5°C, thiourea induced a significant increase in oxygen consumption, which may be expressed by the following mean values : 581.53 per cent for the first group and 627.35 per cent for the second group.

2. Under similar thermic conditions (5°—6°C), the oxygen consumption of the molluscs (third group) treated with thyroid extract showed a considerably lesser increase, the mean being only of 43.76 per cent.

3. In both experimented drugs, the mollusc oxygen consumption tends to return to the starting values 24 hours after the end of the treatment.

The hormonal influences on metabolism in vertebrates and in the first place of substances referred to as opposing the action of these bio-catalysts have not received much attention in the invertebrates.

Ashbell [1] [2] and Duskova [6] reported earlier on the effect of the thyroid hormone on the metabolism in molluscs. The latter found that the administration of thyroid extracts to *Limnea palustris* L. brings about an increase in oxygen consumption.

In dealing with the effect of antithyroid substances on the energy metabolism in molluscs, some investigations have been carried out in

a previous paper by C.A. Picoș and M. Cucerzan [10] in order to detect the effect of thiourea on the oxygen consumption in *Anodonta cygnea* L. at 18°—20°C.

As the utility of supplementary investigations in the field of insufficiently known problems is unanimously accepted, the author of the present paper found it necessary to resume the above-mentioned studies, this time concerning the mollusc *Unio pictorum* L.

The investigations consisted in exposing the animals to cold in order to verify in this phylogenetic stage, too, the fact clearly established in some poikilothermic vertebrates [11] [12] [14] that the effects of thyroid extract and antithyroid substances depend on the thermic conditions under which the experiments are performed.

MATERIAL AND METHOD

The *Unio pictorum* individuals dealt with in our experiments came from the easily flooded waterside of Danube (Crapina, Tulcea distr.).

The animals had been maintained about two months in a large water-filled tank until they were made use of.

Three groups of 3—5 small animals each and varying in weight as little as possible were used : group I, 73 g ; group II, 85 g ; group III, 75.5 g.

As the influence of low-temperature conditions was to be investigated, each group was maintained in the cold (5°—5.5°C) two weeks before the beginning of the determinations.

As the experiments were carried on during the cold season, the above-mentioned temperature was obtained and maintained almost unchanged through a very simple device (Fig. 1), consisting of a large desiccator provided with a side orifice, closed with a stopper carrying a flowing water tube (6). Another tube (5) was disposed in such a manner as to conduct and set permanently in motion the cold water down from a tap to the desiccator passing then the water out of it with a view to permanently maintaining it under hypothermic conditions.

A cylindrical glass flask provided with a fine wire net on its top was put inside the desiccator. Within the cylindrical flask, the group of animals was immersed in two litres of water daily changed.

A thermometer (4) attached to a stand and immersed was provided for the control of the water temperature in the flask which was similar to that passing through the open circuit of the system.

Broadly speaking, the method dealt with was similar for all the groups of animals. Three series of determinations of the oxygen consumption were carried out in each group, corresponding to three successive periods : the period prior to treatment, the period with thiourea (groups I and II) or thyroid extract (group III) treatment and the period following immediately the stopping of treatment. The data of the tables show the number of determinations and the time intervals over which they were made.

Daily doses of the substances whose metabolic action was investigated were administered in water as follows : thiourea, 1 g. per litre of water and thyroid extract, 0.125 g. per litre of water. Before use, the tablets containing this extract were placed in a mortar and ground to a fine powder.

Oxygen consumption in the groups of molluscs has been assayed by a procedure described in a previous paper [10].

The physiological index investigated is defined as ml. of O_2 per kg. of active tissue and per hour.
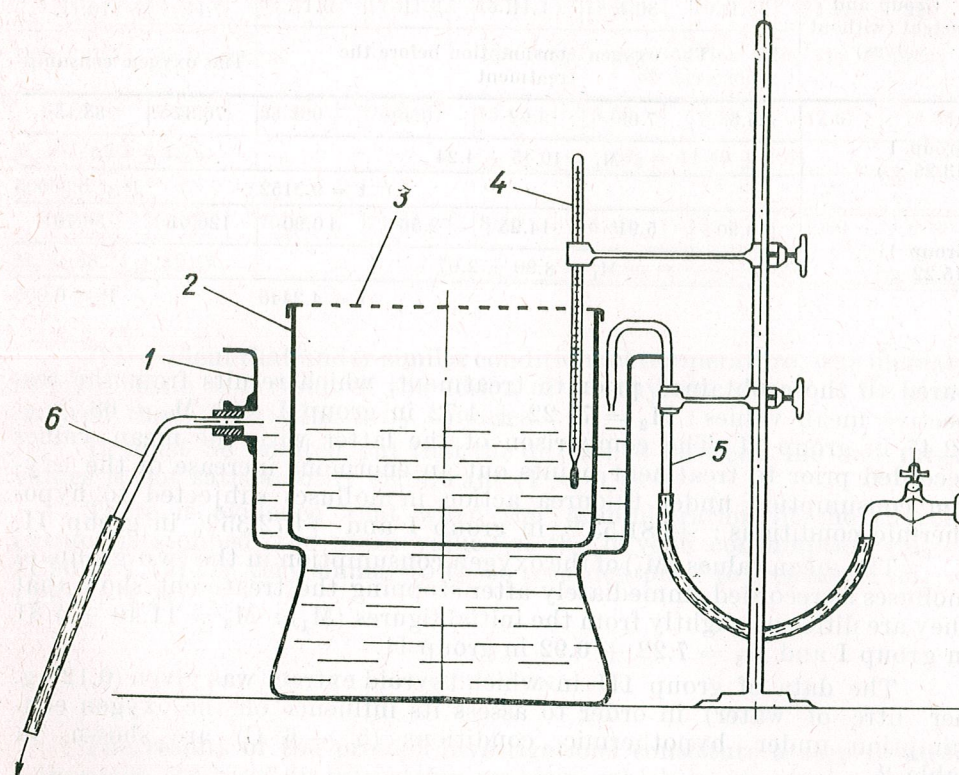
All data obtained were statistically processed.

Fig. 1. — The scheme of the device. (Explanations in the text).

RESULTS

The results of the experiments carried out on groups I and II in which the action of thiourea (1 g. dissolved in 1 litre of water) on the oxygen consumption under hypothermic conditions (+5°) was investigated are presented in table 1.

Examination of table 1 indicates in the first place that under standard conditions, i.e. before the treatment, the mean values of the oxygen consumption in the two groups are expressed in terms of close values : $M_1 = 10.45 \pm 4.24$ in group I, and $M_1 = 8.90 \pm 2.07$ in group II.

One of the values of the oxygen consumption under standard conditions in group I equals zero, fact which is not surprising, since the *Lamellibranchiata* group is known to include optional anoxybiontic animals.

As for the values of the oxygen consumption recorded during treatment (10 days) with thiourea (1 g./l.), a marked increase is found as com-

Table 1

The action of thiourea (1g./l.) upon O_2 consumption (ml./kg./hr.) in *Unio pictorum* L., at 5°C

Group and weight (without valves)	28.I.	30.I.	1.II.	3.II.	5.II.	7.II.	10.II.	11.II.	13.II.	15.II.	17.II.	18.II.	19.II.	20.II.	21.II.	22.II.
	The oxygen consumption before the treatment					The oxygen consumption during the treatment						The oxygen consumption after the stopping of treatment				
Group I (13.25 g.)	25.88	7.69	8.22	10.49	0.0	76.37	88.15	68.67	65.96	53.58	74.64	15.01	5.50	9.35	12.67	14.94
$M_1 = 10.45 \pm 4.24$								$M_2 = 71.22 \pm 4.72$				$M_3 = 11.49 \pm 1.81$				
t = 9.3152; P < 0.00005																
Group II (15.22 g.)	10.90	5.91	14.25	2.56	10.90	126.01	56.70	43.69	44.15	70.10	71.84	8.80	8.40	3.81	8.40	6.70
$M_1 = 8.90 \pm 2.07$								$M_2 = 68.74 \pm 12.47$				$M_3 = 7.22 \pm 0.92$				
t = 4.2440 P < 0.005																

pared to those obtained prior to treatment, which results from the respective mean values : $M_2 = 71.22 \pm 4.72$ in group I and $M_2 = 68.74 \pm 12.47$ in group II. The comparison of the latter with the mean values recorded prior to treatment points out an enormous increase of the oxygen consumption under thiourea action in molluscs subjected to hypothermic conditions : $+581.53\%$ in group I and $+672.35\%$ in group II.

The mean values (M_3) of the oxygen consumption in the two groups of molluscs as recorded immediately after stopping the treatment show that they are differing slightly from the initial figures (M_1) : $M_3 = 11.49 \pm 1.81$ in group I and $M_3 = 7.22 \pm 0.92$ in group II.

The data of group III in which thyroid extract was given (0.125 g. per litre of water) in order to assess its influence on the oxygen consumption under hypothermic conditions (5° — 6° C) are shown in table 2.

It is evident that under similar conditions of temperature, the increase in metabolism as a result of the action of the thyroid extract is far more reduced than that induced by thiourea.

It must be pointed out that the difference between the two mean values is not satisfactorily significant (P < 0.1).

There is evidence that when the administration of the thyroid extract is stopped, the mean values of the oxygen consumption ($M_3 = 11.90 \pm 1.19$) are very similar to those recorded prior to treatment ($M_1 = 12.68 \pm 1.24$).

Table 2

The action of the thyroid extract (0.125 g./l.) upon O_2 consumption (ml./kg./hr.) in *Unio pictorum* L., at 5°–6°C

Group and weight (without valves)	26.II.	27.II.	28.II.	1.III.	3.III.	4.III.	5.III.	6.III.	7.III.	8.III.	9.III.	10.III.	11.III.	12.III.	13.III.	14.III.	15.III.	17.III.
	The oxygen consumption before the treatment					The oxygen consumption during the treatment								The oxygen consumption after the stopping of treatment				
Group III (15.076 g.)	10.41	11.93	16.38	9.98	14.72	13.59	10.41	21.62	26.73	23.94	14.32	13.66	21.62	11.14	8.42	15.58	13.19	11.20
$M_1 = 12.68 \pm 1.24$								$M_2 = 18.23 \pm 2.09$						$M_3 = 11.90 \pm 1.19$				
t = 1.9250; P < 0.10																		

The data of table 2 show that the mean oxygen consumption values as recorded during the treatment ($M_2 = 18.23 \pm 2.09$) are 43.76 per cent greater than those observed before the treatment ($M_1 = 12.68 \pm 1.24$).

DISCUSSION

The results of the present investigations constitute a step towards a thorough study of an interesting problem, which has been also the object of previous researches [10] [11] [12] [14], i.e. the temperature dependence of the metabolic effect of the thyroid extracts and antithyroid substances in poikilothermic animals.

The author of the present paper together with N. Șanta [14] reported in 1954 an important increase in the energy metabolism at 13° — 14°C in tadpoles of *Bombinator pachipus* following thyroid extract administration, while no response was observed at 20° — 21°C.

C.A. Picoș and D. Schmidt [11] noted in a recent paper that in fishes (*Carassius auratus gibelio* Bloch) the metabolic effect of methylthiouracil (an antithyroid substance) is a temperature-dependent phenomenon. Indeed, after methylthiouracil administration a decrease in the oxygen consumption was recorded at 20° —23° C, while at 7° —10° the same substance induced its marked increase. Later investigations on the same species, carried out in collaboration [12], enabled C.A. Picoș to show that under hypothermic conditions (5° —7° C) thiourea also causes an important increase in the oxygen consumption.

Trying to explain the hypermetabolic effects of thioderivates in fishes under hypothermic conditions, we made the assumption that the mentioned substances might exert a direct stimulating action on the tissular respiration, while the function of the thyroid gland remains unchanged.

In fact, as shown by M. Fontaine and co-workers [7], many other scientists are doubtful as concerns the effect of the thyroid gland on the respiratory metabolism in fishes.

We are probably justified in thinking that a much more acceptable view is that expressed by Odette Thibault [15] who claims that the antithyroid substances do not exert an exclusive action on the function of the thyroid gland.

Our investigations on molluscs — animals without thyroid gland — are in agreement with our view according to which we must ascribe to the thioderivates the function of directly stimulating the tissular metabolism. Indeed, in a previously described experiment [10] we have found in *Anodonta cygnea* L. a considerable increase in the oxygen consumption (300 per cent) at 18° —20° C as a result of thiourea administration.

In *Unio pictorum* L. the experimental data of the present paper show a more important increase (about 600 per cent) in the oxygen consumption under low-temperature conditions (5° — 5.5° C) following thiourea administration.

In molluscs the temperature dependence of the metabolic action of thiourea does not express itself through diametrically opposed effects, as it happens in fishes, but through a different amplitude of the same hypermetabolic effect : lower under relative great temperatures, and rising as the temperature gets lower.

The hypermetabolic action of thiourea in molluscs is reversible, since 24 hours after the stopping of treatment this effect is no longer manifest (Table 1).

Various researches on different animals have agreed in showing that the metabolic rate reaches again its normal level after the cessation of antithyroid substances administration.

As for the effect of the thyroid extract on the oxygen consumption in cold-adapted molluscs, personal data show an increase of this metabolic index up to 43.76 per cent.

As previously mentioned, this increase is statistically insignificant. Such lack in significance appears to be even greater if we take into account the supposition that the specific dynamic action of proteins within the thyroid extract may somewhat result in the augmentation of the metabolic rate.

Even if this supposition is to be looked upon as valid, we must admit that thiourea administration induces an increase in oxygen consumption.

This effect can be explained only by the direct stimulation of the tissular metabolism, a fact generally admitted in higher animals having thyroid gland.

Earlier experiments of several authors [1] [2] [5] [6] have shown by means of direct and indirect methods that when molluscs are treated with thyroxine and thyroid extract, the rate of metabolism is greatly increased.

The reversibility of the metabolic action of the thyroid extract is evident, since 24 hours after the stopping of treatment it is no longer recorded.

The facts brought forward in this paper enable us to be in full agreement with those who claim that the fundamental mechanisms by which the thyroid hormone and in the first place the antithyroid substances are acting, are as yet unknown.

CONCLUSIONS

1. A considerable increase in oxygen consumption in two groups of molluscs (*Unio pictorum* L.) — 581.53 per cent in group I and 672.35 per cent in group II — as a result of thiourea administration in water (1 g./l.), under hypothermic conditions (+5°C), is recorded.

2. An increase in the oxygen consumption of only 43.76 per cent in another group of molluscs belonging to the same species, following thyroid extract administration (0.125 g./l.), under similar hypothermic conditions (+ 5 — +6° C), is observed.

3. The hypermetabolic action of both substances is reversible since 24 hours after the end of treatment this metabolic action is no longer manifest.

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TOXINS EFFECT FROM THE CULTURE FILTRATES OF *ERWINIA CHRYSANTHEMI* ON ELECTROPHORETIC PATTERN OF SOLUBLE PROTEINS AND HISTONES FROM GUINEA-PIG LIVER

BY

DRAGOŞ SCRIPCARIU, RADU MEŞTER and ION LAZĂR

In order to explain the mechanism of action of bacterial toxins upon the hepatic cell, the authors undertake an electrophoretic study of proteins and histones from the liver of guinea-pigs treated with filtrates of bacterial culture, injected intraperitoneally. Under the influence of toxin, changes of soluble protein fractions take place in the alpha 1 globulin and alpha 2 group. The disappearance of some fractions from the zymogram, or the appearance of others with electrophoretic properties different from the normal, have been noted. In the case of histones, no modification was recorded in the migration of fractions and their number, as compared with the normal. The intervention of the toxin on the informational mechanism of proteosynthesis is very possible.

Works on the toxic action of the phytopathogenic bacteria are scanty and of a very recent date. Popovici and Lazăr [10] have drawn attention on the pathogenicity and toxicity of the *Erwinia* group species in rabbits and other animals, while Săvulescu and Lazăr [12] have established a relationship between the polyvorousness of *Erwinia* bacteria which induce putrefaction in plants and the degree of nocivity of their toxins. Analysing the toxins of culture filtrates of different *Erwinia* species, Grou and Lazăr [6] [7], and Grou [8] have underlined their nucleoproteic nature ; they are endotoxins which nevertheless exhibit a symptomatology characteristic of exotoxins intoxication.

The intensely noxious action of these filtrates, as well as the nucleoproteic nature of their components, determined us to undertake a detailed study regarding the effects of these toxins on the structure and

cellular functions of laboratory animals in order to elucidate, at least in part, the intimate cytophysiological mechanism of bacterial noxious agents on hepatic and renal cells of guinea-pig.

Our previous investigations [13] have pointed out the fact that the toxin action induces important morphological and histological changes. In renal and hepatic cell tissue of treated animals we noted the appearance of vacuoles, diminution of glycogen content, mitochondrial division into fragments, accompanied by changes of oxydative enzyme activity as well as the increase of hydrolytic enzyme activity with lysosomal localisation, phenomena which finally lead to tissue necrosis.

We have carried out a series of electrophoretic studies in order to explain the action of the filtrate toxin of *Erwinia* culture upon the activity of different enzymes [14—16]. The obtained data render evident the fact that the toxin has an intense inhibitory effect upon the activity of many studied enzymes, leading to changes of the electrophoretic properties or to the appearance of new isoenzymes with characteristic activity and motricity clearly differing from the normal.

The present study exposes data obtained by us on the action of toxic filtrates of *Erwinia chrysanthemi* on the electrophoretic spectrum of soluble proteins and total histones from the guinea-pig liver.

MATERIAL AND METHODS

These investigations were carried out on guinea-pigs having a mean weight of 150 g. The animals were injected intraperitoneally with 1.5 ml. sterile filtrate of *Erwinia chrysanthemi* culture, in 3 doses of 0.5 ml. at 4 hours interval. The animals were sacrified 12 hours after the administration of the last dose. The toxin was obtained according to the method exposed in a previous work [15], and from the liver, a homogenate was prepared according to a technique reported by us [15]. The electrophoretic analysis of proteins was performed with this homogenate.

The electrophoresis was performed during 3 hours at the intensity of 3.12 mA/tube, on gel of polyacrylamide, according to the system of disk electrophoresis [5], using an aliquot of 0.1 ml. supernatant. After electrophoresis, the gel columns were fixed and stained with a solution of 0.5% amido-black 10 B, in aqueous solution of 7.5% acetic acid. Gel differentiation was made by repeated washing in aqueous solution of 9% acetic acid.

For the study of histones their extraction from total liver homogenate was performed according to the method of Agrell et al. [1] and the selective precipitation was made with ammonium reineckate [9]. For electrophoresis we used total extract containing 3 mg./ml. histone in HCl 0.01 N. The electrophoresis was made on gel of polyacrylamide, using the method described by Rebentisch and Debabov [11]. Electrophoresis duration was of 5 hours at an intensity of 6.24 mA/tube. The gels have been stained with amido-black 10 B, solution of 0.5% dye in aqueous solution of 7.5% acetic acid and with aniline black 0.1% dye in aqueous solution of 7.5% acetic acid. Bands differentiation was made by repeated washing in solution of 7% acetic acid.

RESULTS AND DISCUSSIONS

Proteins electrophoresis pointed out certain changes of electrophoretic pattern of hepatic protein fractions. From the normal liver homogenate 16 fractions have been separated. These have been numbered

from anode to cathode according to Webb's technique [18]; with the exception of the first 3 fractions of the albuminous and prealbuminous series, which present variations in width and intesity (with a diminished proteic content), the remaining fractions have a similar width and staining intensity.

Unlike the normal, the electrophoretic pattern of proteins from treated animals present another model; in the globulin fraction alpha 1, the band 7 is absent and between the bands 8 and 9 appears a supplementary band noted 8'. Bands 5 to 10 increase in tinctorial intensity, revealing an increased content of proteins. In the fraction alpha 2 group, an increase of mean intensity of the proteic content was noted in the bands 12 and 13. The characterization, according to protein types, of isolated fractions was made according to Clarke [4] (Fig. 1).

The histones isolated by us from the hydrochloric total extract (without a chemical fractionation of histones types) were separated into 12 fractions. The obtained fractions have approximately the same width and dye intensity, excepting bands 8 and 9, pertaining to the types F_2 and F_3, which exhibit a greater intensity. Localization of types has been made according to Vorobiev et al. [17]. The results obtained revealed no change of these proteins under the influence of the injected bacterial toxins (Fig. 2), but, on the other hand, evinced the changes in the soluble proteins composition, occurring in the hepatic cells under the influence of toxin. The explanation of the appearance or disappearance of certain bands in the electrophoretic pattern of protein is quite difficult. One of the mechanisms of this phenomenon may be the blocking or releasing of certain factors which control this process of protein synthesis. Another explanation may be given by the action exerted by the toxin upon the transfer of certain proteins from the fixed status in different cell structures in solution. Changes induced by the action of toxin on the treated liver homogenate, although important, are insufficient to explain the appearance of numerous new enzymatic fractions in the homogenate of intoxicated animals.

In the literature there is frequently reported that the newly produced enzymes, being in small quantity, are insensitive to the usual dyes for protein, but are developed by the enzymatic activity. The results obtained in the study of histones from liver of treated animals pointed out that this category of proteins is not affected by toxins.

Agrell et al. [1], Black et al. [2] [3] have demonstrated histophotometrically changes of histones concentration of thymus lymphocytes and lymphatic nodes nuclei, under the influence of antigens. The diminution of nuclear histones is accompanied by an increased volume of lymphocytes nucleus and dispersion of chromatina, a phenomenon interpreted as a preimmunitary reaction. Analysing the electrophoresis of histones in mice treated with tetanic toxin, Agrell et al. [1] did not record significant changes of their electrophoretic spectrum. The authors explained the histochemically observed phenomenon of histone solubilisation on the chromatine which lead to a change of histone/DNA ratio, by a migration of nuclear histone towards the cytoplasm. This solubilisation does not involve a change of the histonic composition in the whole cell, but allows the release of certain chromosomal DNA areas from

histonic blocking. The liberation of several chromosomal loci lead to the activation of certain genes, which, through the messenger RNA, takes part, in the cases described by the authors, in antibody proteins synthesis.

Simultaneously with the redistribution of histones, a redistribution of DNA between the soluble and insoluble phase takes place, leading
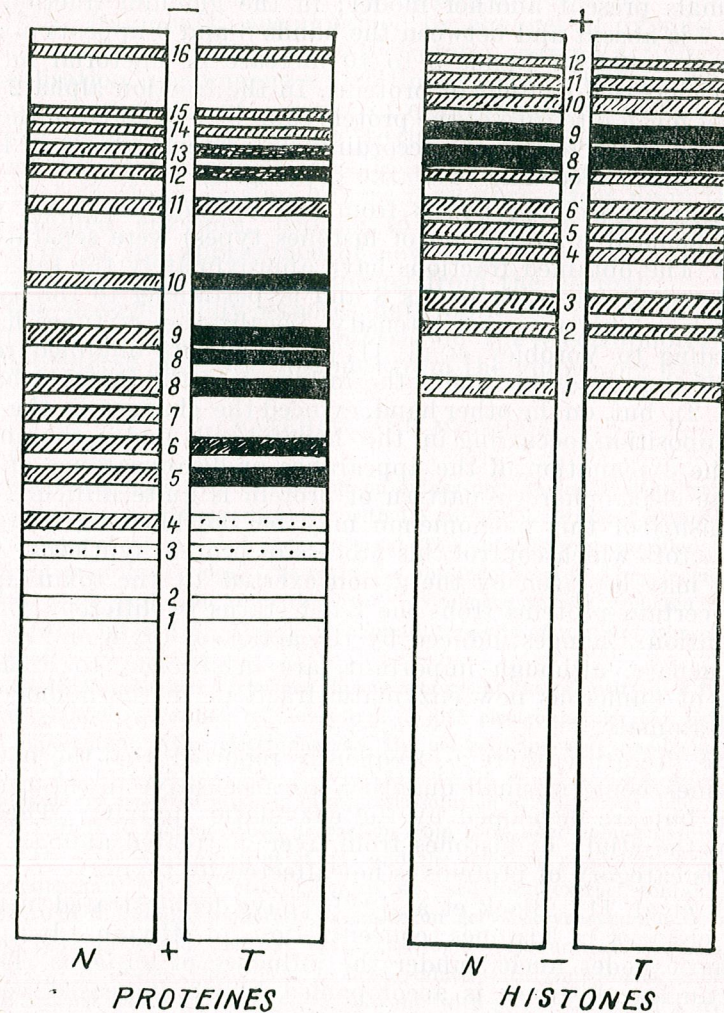

Fig. 1. — Electrophoretic picture of proteins from liver homogenates of normal (N) and treated (T) animals.

Fig. 2. — Electrophoretic picture of histones extracted from the liver of normal (N) and treated (T) animals.

to a change in its genetic activation. The schedule elaborated by Agrell et al. [1] may be considered as an explanation of the appearance of new enzymatic fractions in the organs of treated animals. The histophotometrical, biochemical and electron microscopic verification of the repe-

tition of this phenomenon in the liver treated with toxic filtrates of E. chrysanthemi culture, will form the object of further investigations.

CONCLUSIONS

1. As a result of the treatment with toxic culture of *Erwinia chrysanthemi*, there is a change of electrophoretic spectrum of the liver soluble proteins. In the globulin fraction of treated animals (alpha 1 group), band 7 disappears and an intermediary band appears, between the bands 8 and 9, marked 8′. Bands 5 to 10 obtained from the liver homogenates of treated animals increase in tinctorial intensity.

2. Hepatic histones are not modified under the influence of toxin.

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EFFET DE L'EAU LOURDE SUR LES RAPPORTS ISOTOPIQUES $^{13}C/^{12}C$ DU CO_2 EXHALÉ PAR LES RATS *

PAR

FLORENTINA MOSORA

The isotopic ratio $^{13}C/^{12}C$ (expressing per mil differences from a standard) of CO_2 exhaled by rats showing symptoms of deuterium intoxication increases by 40% in the period of high metabolic rate and decreases by 50% when the metabolic rate falls below normal. The results obtained demonstrate that the changes of this isotopic ratio are due to the modifications induced by heavy water in the metabolic rate.

On sait que les rapports isotopiques du carbone et d'autres éléments, dont l'oxygène, sont susceptibles de variations significatives, aussi bien dans la matière cosmique que sur la Terre [8]. Dans le domaine biologique on a déjà démontré qu'il existe des fractionnements. Par exemple, chez les plantes, Baertschi [1] [2], Park et Epstein [7] ont révélé l'existence d'un fractionnement isotopique pour le rapport $^{13}C/^{12}C$ au cours de la photosynthèse. Ils ont aussi montré que la partie lipidique des plantes est plus riche en ^{12}C que les autres parties [6]. Les fractionnements peuvent être dus à des différences dans les taux de réaction ou de diffusion, ou à des différences dans les constantes d'équilibre. Sur ce sujet ont paru très peu d'études de recherche systématique, mais les données observées s'accordent avec l'hypothèse que les isotopes légers parcourent les voies métaboliques plus rapidement que les isotopes lourds.

Parmi les observations intéressantes auxquelles on a accordé une attention particulière se trouve l'effet léthal du deutérium sur la plupart des organismes. Toutes les recherches montrent que, à une concentration

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d'eau lourde inférieure à celle qui provoque la mort des organismes vivants, le D_2O est la cause de plusieurs modifications métaboliques.

Le but de la présente Note est d'examiner l'effet des modifications métaboliques, provoquées par l'eau lourde, sur les rapports isotopiques $^{13}C/^{12}C$ du CO_2 exhalé par les rats.

Les travaux ont été exécutés au moyen d'un spectromètre de masse (Varian MAT-système CH5) à focalisation unique et à double collecteur, permettant de mesurer des différences de rapports isotopiques avec une précision d'environ 3.10^{-2} pour mille. Nous avons recueilli des échantillons de CO_2 chez un groupe de 12 rats mâles de même race et de même âge et de poids égal (250 g). Le gaz exhalé par les rats — enfermés dans une enceinte dont l'air ambiant était exempt de CO_2 atmosphérique — était séché sur P_2O_5 et recueilli ensuite dans un piège à azote liquide.

On a établi au préalable la composition isotopique relative (δ) pour le rat normal. La valeur de δ indique la différence, en pour mille, entre le rapport $^{13}C/^{12}C$ de l'échantillon et celui du standard qui, dans ce cas, provenait d'une bonbonne de CO_2 pure :

$$\delta\ (^o/_{oo}) = \left[\frac{(^{13}C/^{12}C)_{\text{échantillon}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1 \right] \cdot 1000$$

La valeur normale de δ pour chaque rat a été établie faisant la moyenne pour les valeurs obtenues journellement, pendant 10 jours (les conditions d'expérience, pour tous les rats, ont été les mêmes : les prélèvements de CO_2 ont été faits à jeun). La composition isotopique relative (δ) varie pour le rat normal entre $-1,50$ et $-1,85$. Notons, en outre, que le standard utilisé par nous n'a pas été déterminé relativement au standard international PDB et la mesure de δ n'a pas été corrigée pour ^{17}O parce que le but de ce travail était d'observer un éventuel effet de l'eau lourde sur δ, établi dans nos conditions expérimentales.

Pour examiner l'effet de l'eau lourde on a observé la variation de δ (valeur normale), établie pour chaque rat, après des injections de 0,1 ml/g de D_2O par jour.

Dans ces conditions la mort survient après 5—6 jours, quand le remplacement de l'eau du corps par le D_2O atteint 35—40 pour cent. Toutefois il est déjà établi [3] [4] [5] [9] que le remplacement de 15 pour cent jusqu'à 20 pour cent de l'eau du corps par de l'eau lourde provoque chez le rat un état d'hyperexcitabilité qui correspond à une augmentation de 20 pour cent du niveau du métabolisme normal. A la suite d'une substitution de 35—40 pour cent s'installe l'étape préléthale, les animaux refusent de manger et l'on constate une baisse de leur métabolisme de 80 pour cent par rapport à la normale.

Les résultats obtenus par nous indiquent que la valeur moyenne de δ subit en fait une augmentation de 40 pour cent ($-2,34$) dans la période caractérisée par le métabolisme élevé et une diminution de 50 pour cent ($-0,84$) pour la période du métabolisme très bas.

En résumé, il ne fait pas de doute que les différences de la composition isotopique relative (δ), en ce qui concerne le rapport $^{13}C/^{12}C$ du CO_2 exhalé par les rats, provoquées par l'influence de l'eau lourde, sont l'effet d'une modification du métabolisme. Le mécanisme intime est inconnu

et il est trop tôt pour tenter de proposer une explication précise de ces effets nucléaires. Quant à l'interprétation qui implique probablement du moins partiellement, des effets cinétiques en rapport avec le cycle de Krebs, elle doit attendre les résultats de nouvelles expériences. Mais il semble toutefois que l'on puisse suggérer qu'il s'agirait là d'un phénomène qui aurait lieu aussi à l'état normal et par conséquent les variations des rapports isotopiques en cause, d'un individu à l'autre, suivraient les différences métaboliques entre les individus.

REMERCIEMENTS

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EFFECTS OF SINUSOIDAL CURRENTS WITH 16—25,000 Hz FREQUENCIES ON THE MOTION AND INTEGRITY OF SOME CILIATES (*STYLONYCHIA* AND *PARAMECIUM*)*

BY

C. PORTELLI

The present work deals with the modifications produced in a number of ciliates (*Stylonychia* and *Paramecium*) submitted to various voltages and frequencies of the sinusoidal current. Four types of effects have been found, depending on the frequencies and voltages of the current used : rotation of cells around their posterior pole ; reversed direction of their motion ; paralysis of the motion and disruption of the cell membrane.

Under a light microscope there have been observed changes, induced by sinusoidal currents with 16—25,000 Hz frequencies, in the aspect and mobility of ciliates of the species *Stylonychia* and *Paramecium*.

MATERIAL AND METHOD

1. A thin plate of transparent plexi in which a cavity was hollowed out, about 1 cm². in surface and 3 mm. or so in depth.
2. A generator of sinusoidal currents with frequencies between 16 and 25,000 Hz, whose amplitude was adjustable.
3. A current amplifier.
4. A culture of *Stylonychia* and *Paramecium*.
5. A light microscope.

A drop of ciliate medium was taken with a pipette and put into the cavity of the plexi plate. The motion of the ciliates was observed under the microscope after sinusoidal currents had been applied for a very short interval by means of two electrodes. The motion
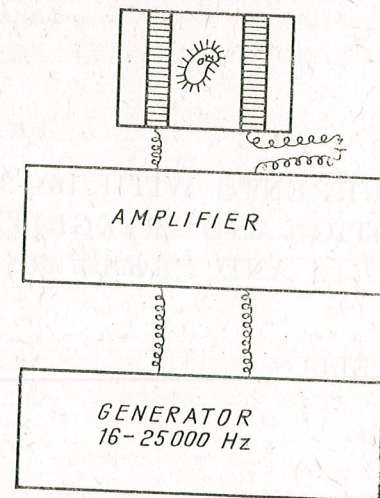
Fig. 1. — Scheme of the device.

changes of the ciliates were noted down along with the frequency and tension values of the sinusoidal current that produced them.

The experiment was performed at a constant temperature (22°C) and pH [7].

RESULTS

Depending on the frequency and voltage applied, four types of effects have been found for *Stylonychia* :

1. rotation of cells around their posterior pole ;
2. reversed direction of their motion, the posterior pole coming in front ;

Fig. 2. — The voltage threshold as a function of frequency of sinusoidal electric currents for *Stylonychia*.
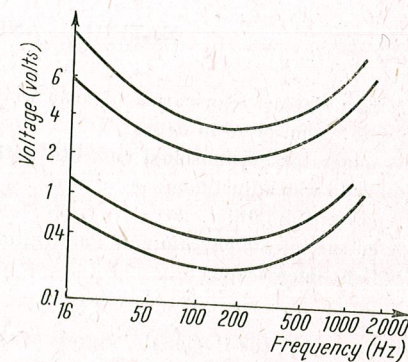
3. paralysis of the motion ;
4. disruption of the cell membrane.

The four types of effects appeared in the order mentioned above, as a consequence of a progressive increase in the voltage of sinusoidal currents.

The curves representing the voltage threshold indispensable for the obtaining of one of these effects showed, each of them, a minimum between 50 and 250 Hz. None of the present effects was found at frequencies above 5,000 Hz.

Paramecium cells exhibited under the action of sinusoidal currents only an effect of paralysis of the active motion, which was recorded as a curve showing also a minimum at frequencies between 50 and 250 Hz.
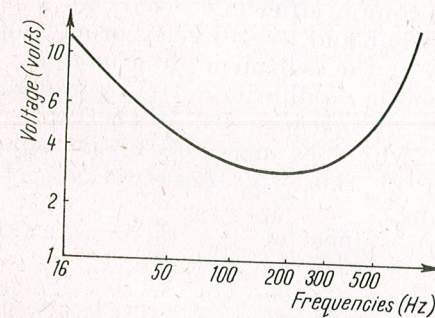

Fig. 3. — The voltage threshold as a function of the frequency of sinusoidal electric currents which produces an effect of paralysis in *Paramecium*.

DISCUSSION

Our data point out an increased sensitivity of the cell at frequencies of 50—250 Hz, which seems to be in connection with an exciting resonance. A. Monnier described a similar resonance for the nerve [5].

An electrical impulse must have a certain duration and intensity in order to be able to provoke an excitation. Between the duration and the intensity of the threshold impulse there is an inversely proportional ratio. The exciting impulse must show also a certain velocity of variation, as otherwise it becomes ineffective through an accomodation phenomenon.

The minimum threshold voltage of 50—250 Hz is probably due to two factors :

1) At high frequencies, the duration of the stimulating impulse diminishes, thus producing an increase in the intensity threshold.

2) At low frequencies, the slope of the stimulating impulse is smaller, which also results in an increase of the intensity threshold by a phenomenon of accomodation.

On the other hand the responses of ciliates are various, depending on the voltage and frequency of the current applied.

A. Grebecki [2] and Stanislav Dryl [1] also showed that *Paramecium* responds differently to a continuous current, as a function of voltage. Thus, at a 0.1—0.2 mA voltage, the *Paramecium* swims with the anterior pole towards the anode, while at 0.3—0.5 mA it moves with its posterior pole towards the cathode.

Yutaka Naitoh and Roger Eckert [6] used microelectrodes to investigate the electrical potentials of *Euplotes* and showed that if an *Euplotes* cell is subjected to a light stimulus at its anterior pole, a depolarization of the cell membrane is produced and its cilia turn for forward swimming. In case it is the posterior pole of the cell which undergoes the action of a light stimulus, the cell membrane is hyperpolarized and the cilia find their way for swimming backwards. Thus the direction of the cilia is determined by the polarization of the cell membrane and the membrane exhibits in its turn several electrical levels with a functional role. Under the influence of sinusoidal currents, a gradual depolarization of the cell membrane is produced. Similar modifications were observed in the nerve [4]. A depolarization of maximum efficiency occurs at a 50—250 Hz frequency of the sinusoidal current and it is directly proportional to its voltage. But the depolarization of the cell membrane modifies its permeability as well as its hydro-osmotic equilibrium. Hence probably, as a consequence, the breaking of the *Stylonychia* cells, at a certain voltage and frequency. A question arises : why does such a disruption appear only in *Stylonychia* cells and not also in those of *Paramecium*? This difference could be due to the following :

a) *Stylonychia* has a smaller resistance of the membrane than *Paramecium* ;

b) *Stylonychia* is 5 times longer than *Paramecium*, so that when an electric tension is applied, the tension difference between the anterior and posterior pole is 5 times greater in *Stylonychia* than in *Paramecium*.

By comparing the curves of sensitivity to various voltages and frequencies, we can see the difference between the two ciliates. This suggests the possibility of utilizing sinusoidal currents for determining certain parameters of cellular excitability as *rheobasis* and *chronaxia*. For example, it is possible to determine a relative rheobasis by establishing the smallest tension of sinusoidal current with the lowest frequency which is still able to induce a modification in the motility of the ciliate, taken as a test. It is possible to determine a chronaxia too, by establishing the highest frequency of sinusoidal current capable of producing the selected change when the current voltage amounts to the double of the rheobasis value. In this case chronaxia is $C = \dfrac{1}{2f}$, where f is the frequency of the sinusoidal current.

The phenomenon of selective breaking of cells by sinusoidal currents will perhaps find in the future a practical utilization in therapeutics.

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