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SOMMAIRE

SUR *SARCOPHAGA (AETHIOPISCA) AFRA* SENSU ZUMPT 1972 ET LA DESCRIPTION D'UN NOUVEAU GENRE AFROTROPICAL (*DIPTERA, SARCOPHAGIDAE*)

ANDY Z. LEHRER

A new genus (*Hadashophalla* n. gen.) and a new species (*Hadashophalla tautella* n. sp.) for the afrotropical Sarcophaginae are described.

Dans ses études taxonomiques sur les Sarcophagines éthiopiennes, Zumpt a considéré que la plupart des caractères morphologiques «non-hypopygial» ont une dominance plus grande sur ceux liés de l'armature génitale mâle des taxons. D'ici proviennent, tout d'abord, sa conception générale que les nombreuses espèces de la sous-famille des Sarcophagines appartiennent au genre géant «*Sarcophaga* Meigen» et, puis, l'hétérogénéité structurale qui se trouve dans la composition de ses «sous-genres» fondamentalement artificiels. En même temps, nous avons constaté que le complètement des diagnoses des espèces décrites par lui avec l'illustration de leur génitalie est plus formel et particulièrement déroutant par son incompréhension.

En plus, son indifférence concernant la valeur des structures de ce complexe d'organes – qui assure la perpétuation des espèces et, par son grand rôle biologique, présente la garantie d'une variabilité seulement dans les limites exceptionnellement petites – l'a déterminé à modifier, dans ses différents travaux, l'image de la génitalie de la même espèce ou de présenter les génitalies des espèces différentes comme variations individuelles du même taxon. Hormis ces choses, il a fait de nombreuses interprétations fausses des dessins des autres auteurs et, surtout, de ceux des espèces de Curran (1934) et Rohdendorf (1963), sans consulter d'une manière solide les types de celles-ci et sans pouvoir faire une comparaison réelle entre leurs dessins et les images fantaisistes réalisées par ses collaborateurs.

Dans sa monographie de 1972 on peut rencontrer beaucoup de confusions taxonomiques déterminées par son incompatibilité pour les techniques microscopiques même habituelles avec lesquelles se réalisent les préparations des armatures génitales mâles et s'obtiennent les dessins compréhensibles sur leurs structures. Parmi celles-ci nous avons trouvé aussi le cas de l'espèce «*Sarcophaga afra*» sensu Zumpt (nec Curran), qui a été introduite dans le «sous-genre *Aethiopisca* Rohdendorf», avec quelques espèces très éloignées au point de vue systématique.

C. H. Curran (1934:18) a décrit pour la première fois son espèce nouvelle *Sarcophaga afra* d'après 7 ♂♂ et 9 ♀♀ du Zaïre. Les caractères tout à fait originaux de sa génitalie mâle (fig. l, A), avec l'ensemble des caractères généraux, ont obligé Rohdendorf (1963:2 et 12) de créer pour celle-ci le genre monospécifique *Aethiopisca*. Zumpt (loc. cit.) a adopté ce genre avec le rang de «sous-genre» et,

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puis, J. P. Dear (1980:810) l'a utilisé pour *Sarcophaga afra* sensu Zumpt 1972, *Sarcophaga aenigma* Rohdendorf et *Sarcophaga gertrudae* Zumpt, dans la vision de l'auteur précité.

Comparant les dessins du Curran avec ceux du Zumpt pour l'armature génitale mâle de l'espèce *Sarcophaga afra* Curran, nous avons constaté qu'ils ne sont ni même un peu pareils, mais qu'il sont extraordinairement dissemblables. Leurs différences ne peuvent, en tout cas, être interprétées comme variations individuelles. Elles sont en réalité l'expression des divergences structurales incontestables. Ces observations nous ont conduit à la conclusion que Zumpt a commis une profonde erreur en ce qui concerne son identification et que, vraiment, nous sommes en face d'un genre et d'une espèce nouveaux pour la faune afrotropicale: *Hadashophalla* n. gen. et *Hadashophalla tautella* n. sp.

Bien que Curran (1934:18) n'ait pas décrit les composantes génitales de *Sarcophaga afra*, Rohdendorf (1963:12) interpréta son dessin assez schématique, en disant que le distiphallus a «stark entwickelten membranösen Apikalteil der Paraphallus, sklerosierte, lateralwärts gerichtete Ventralapophysen des Basalteils, kurze Styli und schwach entwickelte Membranalloben». Zumpt (loc. cit.) n'a pas perdu l'occasion d'exprimer son doute injustifié à l'égard des opinions de Rohdendorf et il a fait l'affirmation suivante, au moins, sceptique: «whether the homology of the parts of the phallosome indicated by ROHDENDORF is correct, remains to be proved». D'ici résulte avec la plus grande clarté que Zumpt n'a vu et n'a vérifié jamais le type de l'espèce du Curran et que son identification a été hasardée.

Genre **Hadashophalla** n. gen.

= *Sarcophaga (Aethiopisca)* sensu Zumpt 1972, Explor. Parc Nat. Virunga, **101**:40 - syn. n.; sensu Dear 1980, Catalogue of afrotropical Diptera, 810 - syn. n.

= *Sarcophaga (Sudafricana)* sensu Dear 1980, Catalogue of afrotropical Diptera, 810 - syn. n.

Espèce-type. Hadashophalla tautella n. sp. (=*Sarcophaga afra* sensu Zumpt 1972 [nec Curran 1934]; identification erronée).

Diagnose du genre. Bien que Zumpt n'ait pas décrit correctement son espèce *Sarcophaga afra*, nous pouvons admettre que ce genre a 4 macrochètes dorso-centraux postsuturaux, les propleures poilues et les ailes transparentes, sans tâches. Mais son type de structure de l'armature génitale est totalement différent de celui du genre *Aethiopisca* Rohdendorf 1963. Les cerques sont relativement plus longs et ondulés. Les paralobes sont très longs et plus ou moins étroits. Distiphallus est relativement petit. La partie basale du paraphallus est courte et avec (probablement) les lobes paraphalliques donne l'aspect d'une bottine. La partie apicale du paraphallus est longue, membraneuse et très ondulée à la marge inférieure. Les prégonites sont étroits et bifides aux sommets; les postgonites sont très larges et ont un sommet mince, long et courbé.

Composition du genre. Hadashophalla tautella n. sp.

Hadashophalla tautella n. sp.

= *Sarcophaga (Aethiopisca) afra* sensu Zumt 1972, Explor. Parc Nat. Virunga, **101**:89, fig. 39 (nec Curran 1934; nec Rohdendorf 1963; identification erronée); sensu Dear 1980, Catalogue of afrotropical Diptera, 810 - syn. n.

MÂLE

Les caractères morphologiques «*non-hypopygial*» (Zumpt, loc. cit.) de *Sarcophaga afra* présentés par Zumpt sont très sommaires et pas significatifs. Il mentionne que les propleures sont poilues, les tibias postérieurs pourvus d'une longue pilosité sur les deux parties ventrales, l'abdomen a un tomentum cendré, les dessins abdominaux en damiers et bien définis, sans les macrochètes médio-postérieurs sur le tergite III.

Armature génitale: fig. 1, B (d'après Zumpt). En comparaison avec *Aethiopisca afra* (Curran, 1934) (voir, fig. 1, A; d'après Curran), les cerques de celle-ci sont longs et ondulés; ils présentent une courbure dorsale large dans le tiers apical et un sommet allongé, oblique, ondulé et aigu; les paralobes sont relativement très longs et étroits. La partie basale du paraphallus est fortement sclérifiée et, avec (probablement) les lobes paraphalliques, a une forme d'une bottine; elle n'a pas d'apophyses latérales grandes qu'à l'espèce *Aethiopisca afra* (Curran).

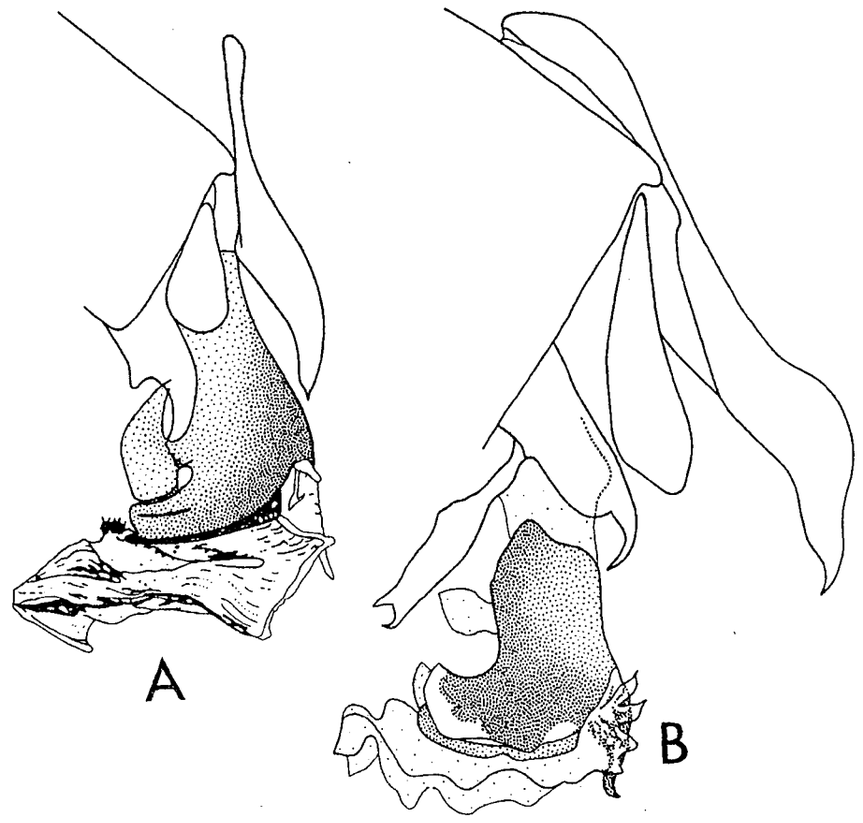

Fig. 1 – Armatures génitales mâles de *Aethiopisca afra* (Curran, 1934; A, d'après Curran, un peu modifiée) et de *Hadashophalla tautella* n. gen., n. sp. (B; d'après Zumpt, un peu modifiée).

La partie apicale du paraphallus est longue, membraneuse, sans une forme bien définie, mais dans sa région proximale elle est pourvue de 5 formations membraneuses, foliacées et déployées. Les styles ne s'observent pas, mais nous supposons qu'il sont très courts. Les lobes membraneux sont assez bien développés, mais membraneux, plus ou moins ovalaires et orientés ventralement. Les prégonites sont longs, étroits et bifides aux bouts; les postgonites sont très larges, longs et ont le sommet long, mince et courbé.

Terra typica. Zumpt a mentionné 4 ♂♂; 1 ♂ originaire de Togo, étant gardé dans les collections de l'Institut Sud-Africain de Recherches Médicales de Johannesburg et 3 ♂♂ colligés au Sudan, qui se trouvent dans les collections du Musée Zoologique de Berlin. Malheureusement nous ne connaissons exactement quel mâle a fournis l'armature génitale qui a été à la base des dessins de Zumpt, pour le désigner comme holotype. Nous croyons que celui-ci provienne de Togo.

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SUR LES GRÉGARINES (PROTOZOA, SPOROZOA) PARASITES CHEZ *RIVULOGAMMARUS* (CRUST. AMPH.) DES RUISSEAUX DE MONTAGNE EN ROUMANIE

DOINA CODREANU-BĂLCESCU

There are presented the Gregarines (Protozoa, Sporozoa) parasitising in the gut of the amphipode (Crust.) *Gammarus (Rivulogammarus) balcanicus montanus* St. Karaman, 1929 living in mountain streams from Southern Carpathians. Four new species belonging to the genus *Cephaloidophora* are described: *C. rectangularis* n.sp., *C. crassa* n.sp., *C. dissimilis* n.sp. and *C. stentor* n.sp. It is recorded for the first time by gammarids from Romania, as well as in the s.g. *Rivulogammarus*, the species *Uradiophora longissima* (Siebold, 1839) Poisson, 1924 and *Cephaloidophora gammari* (Diesing,1859) nov. comb. The polyparasitic state of the Gregarine parasites in gammarides is discussed.

Les crustacés amphipodes constituent, dans l'eau douce et salmastre de Roumanie, un groupe complexe, tant du point de vue de l'origine, que de leur composition, ayant une importance majeure dans les chaînes trophiques des écosystèmes aquatiques. En poursuivant les recherches sur leurs Grégarines, considérées comme facteurs possibles limitatifs naturels dans les populations de diverses biocénoses, on étudie les espèces parasites chez *Gammarus (Rivulogammarus) balcanicus montanus* St. Karaman, 1929, habitant les ruisseaux de montagne du bassin supérieur de la Prahova (Massifs de Bucegi et Gârbova) et la vallée de la Bârsa (Massif Piatra Craiului), dans les Carpates Méridionales. Il s'agit de 5 espèces du genre *Cephaloidophora* Mavrodiadi, 1908, dont 4 nouvelles et l'autre, pour la première fois citée chez cette hôte, en Roumanie, également qu'une espèce appartenant au genre *Uradiophora* Mercier, 1912, que nous venons de décrire ci-dessous.

Des échantillons répétés des gammares ont été prélevés pendant les 20 dernières années (les mois d'avril-novembre), leurs intestins disséqués et les grégarines endoparasites examinées sur le vivant (solution de Ringer, colorations vitales) ou fixées aux vapeures osmiques. On a pratiqué des mesures, dessins à la chambre claire et photographies en contraste de phase.

I. Genre *Cephaloidophora* Mavrodiadi, 1908

(Fam. des CEPHALOIDOPHORIDAE Watson-Kamm, 1922)

Eugrégarines parasites dans l'intestin des crustacés. Stades intracellulaires au début du développement. L'épimérite réduit au mucron. Syzygies fronto-caudales précoces, dont les partenaires peuvent être différents. Les espèces du genre susceptibles d'importantes variations morphologiques.

1. *Cephaloidophora gammari* (Franzius, 1848) nov. comb.

Syn: *Gregarina gammari* Franzius, 1848; *Cephaloidophora echinogammari* Poisson, 1921; *Rotundula gammari* Goodrich, 1949

Céphalins isolés, de 35-75 μ de longueur (exceptionnellement 100 μ, fig. 1) ont une forme caractéristique ovale, légèrement allongée, portant antérieurement

le mucron, sous forme d'un bouton proéminent, persistant. Le protomérite conformé en une calotte est séparé par un septum convexe, de côté d'un deutomérite ovale, visiblement plus allongé chez des jeunes individus. Le noyau, pourvu d'un gros nucléole, a une localisation variable dans le deutomérite. Les syzygies fronto-caudales se forment de bonne heure (fig.2); il y a des cas où le satellite peut être plus petit que le primite. Les partenaires continuent leur croissance, devenant beaucoup plus volumineux et chargés d'enclaves polysaccharidiques. Les syzygies ont une longueur totale de 70-130 μ, elles glissent dans des directions opposées, en exécutant des mouvements de raccourcissement et d'épaississement du deutomérité.

Les indices morphométriques des gamontes en syzygies adultes:

TL	LE	LP	LD	LP:TL	WP	WD	WP:WD	N
64μ	5μ	15μ	45μ	1:4,2	24μ	33μ	1:1,37	8μ

En Roumanie on a trouvé cette espèce dans l'intestin antérieur et moyen de *Rivulogammarus* de Valea Cerbului (M. Bucegi), Tufa, Sipa (M. Gârbova), vallée de la Bârsa (M. Piatra Craiului), le pourcentage d'infestation étant plus de 50%, et souvent en association avec *Uradiophora longissima* et d'autres espèces de *Cephaloidophora*, surtout *C. rectangularis* et *C. crassa*. Il s'agit d'une espèce à large distribution, citée chez d'autres gammarides, en Europe: *Gammarus pulex* (France, Angleterre) (7) (9), *G.roeselii* (Allemagne) (4) et en Amérique du Nord: *G. fasciatus* (6).

2. *Cephaloidophora rectangularis* n.sp.

Des stades intracellulaires arrondis (∅ =9 μ). Les plus jeunes stades observés dans la lumière intestinale sont des céphalins libres (L=15μ; l=6μ), munis d'un mucron d'env. 1μ dehauteur. Le protomérite globuleux-tassé, représente 1/4 de la longueur totale, étant un peu plus étroit que le deutomérite rectangulaire-allongé. Leur cytoplasme renferme peu d'inclusions, ayant l'aspect hyalin. Tels individus solitaires,atteignant env.36μ delong,commencent à s'associer en syzygies fronto-caudales, en continuant leur croissance et l'accumulation des reserves. Les gamontes d'une syzygie adulte (fig. 3) sont généralement égaux, le primite pouvant être un peu plus grand que le satellite. Ils ont une forme caractéristique rectangulaire-allongée et chaque gamonte présente un protomérite conformé en cube, d'une largeur à peu près égale à celle du deutomérite et offrant à son pôle libre un mucron lenticulaire saillant, très hyalin par rapport au reste du corps, fortement refringent. Le deutomérite long-rectangulaire a son bord caudal légèrement excavé et renferme le noyau arrondi, muni d'un nucléole important, dans sa moitié supérieure, près du septum convexe postérieurement.

Les indices morphométriques des gamontes en syzygies adultes:

TL	LE	LP	LD	LP:TL	WP	WD	WP:WD	N
51μ	3,4μ	13μ	35μ	1:4	18μ	20μ	1:1,1	8μ

L'espèce a été trouvée associée avec *C. gammari* et *U. longissima* chez des *Rivulogammarus* provenant de la vallée de la Bârsa et Valea Cerbului et dans des infestations singulières à Valea Căşăriei (M.Gârbova).

3.*Cephaloidophora crassa* n.sp.

A l'exception des sporadins isolés, arrondis (dimensions maximums 63μ ×57μ), ayant le bord caudal invaginé et munis antérieurement d'un goulot très

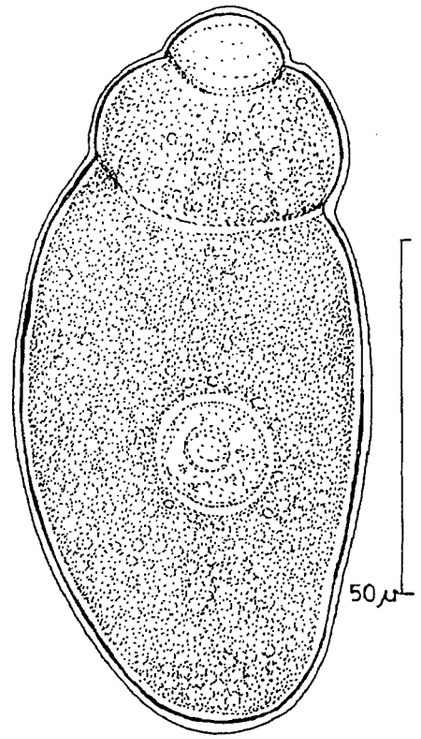

Fig. 1. – *Cephaloidophora gammari* (Franzius, 1848) nov. comb.-gamonte solitaire, taille maxima.

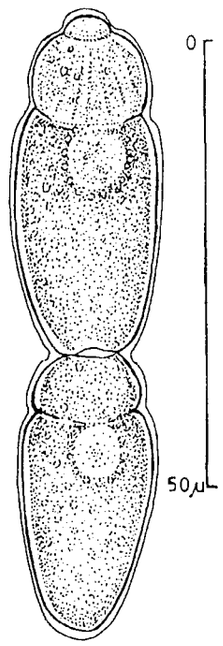

Fig. 2. – *Cephaloidophora gammari* (Franzius, 1848) nov. comb.-syzygie très jeune.

opaque, la plupart des stades rencontrés sont des svzygies fronto-caudales, d'une longueur totale moyenne d' env. 100μ (exceptionnellement 130μ). Elles sont constituées des gamontes inégaux comme forme et taille (fig.4). Le primite est plus gros, globuleux et son contenu est beaucoup plus dense relativement à celui du deutomérite. Son épimérite est pressé dans le protomérite, qui, à son tour, s'enfonce dans le deutomérite, prenant l'aspect d'un col roulé, avec le septum fortement voûté vers le deutomérite, tandis que son bord frontal reste aplati. Le satellite est plus petit, cordiforme, avec l'extrémité caudale plus ou moins arrondie, parfois excavée. Les noyaux ont une position centrale dans le deutomérite.

Les indices morphométriques des gamontes en syzygies adultes:

	TL	LP	LD	LP:TL	WP	WD	WP:WD	N
primite	55μ	17μ	38μ	1:3,2	39μ	60μ	1:1,5	16μ
satell.	48μ	21μ	27μ	1:2,3	33μ	37μ	1:1,1	8μ

On a trouvé cette espèce dans des infestations singulières chez des hôtes du ruisseau Babei (Cumpătu, Sinaia) et asociée avec *C. gammari* et *U. longissima* dans Valea Cerbului.

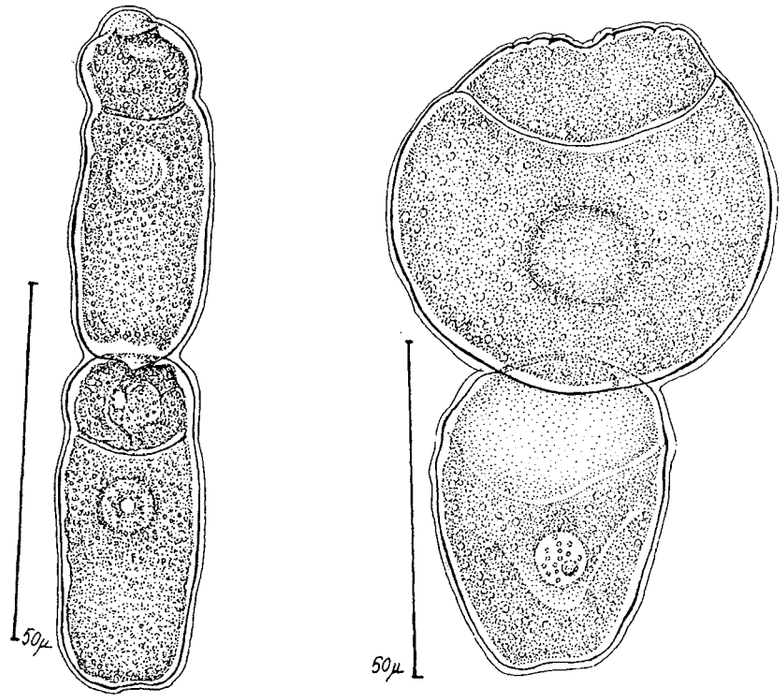

Fig. 3. – *Cephaloidophora rectangularis* n.sp.-syzygie adulte.

Fig. 4. – *Cephaloidophora crassa* n.sp.-syzygie adulte.

4. *Cephaloidophora dissimilis* n.sp.

Grosses syzygies de 120μ-180μ de longueur totale, constituées des gamontes à conformation et taille tout à fait différentes (fig. 5). Le primite est claviforme, légèrement dilaté aux extrémités, plus important que le satellite, beaucoup plus petit, condensé, presque globuleux, Les deux ont des épimérites lenticulaires et leurs protomérites arrondis sont enfoncés dans les deutomérites, produisant sur le vivant des zones concentriques antérieures, plus foncées que le reste du cytoplasme, contenant de fines inclusions denses. Les noyaux, munis d'un gros nucléole, sont situés chez le primite dans la moitié inférieure du deutomérite, et dans son milieu chez les satellites.

Les indices morphométriques des gamontes en syzygies adultes:

	TL	LP	LD	LP:TL	WP	WD	WP:WD	N
primite	130μ	20μ	110μ	1:6	34μ	55μ	1:1,6	17μ
satell.	51μ	20μ	31μ	1:2,5	38μ	52μ	1:1,4	20μ

L'espèce est plus rare (3-5% taux d'infestation) chez des hôtes dans Valea Cerbului.

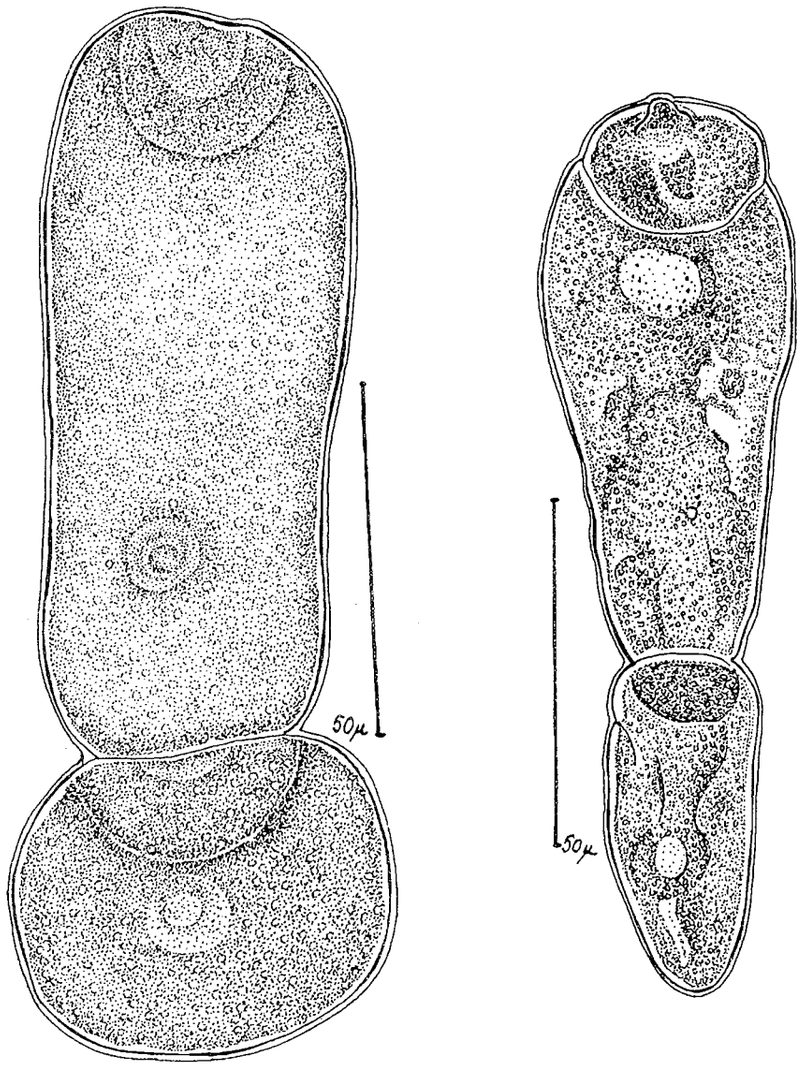

Fig. 5. – *Cephaloidophora dissimilis* n.sp.-syzygie adulte.

Fig. 6. – *Cephaloidophora stentor* n.sp.-syzygie adulte.

5. *Cephaloidophora stentor* n.sp.

On rencontre dans la lumière de l'intestin moyen très rarement des sporadins solitaires (L = 40-50μ; l = 16-20μ), peu différents comme forme et mesures, ainsi que des jeunes syzygies (LTS moyenne = 70 μ), dans lesquelles s'associent des

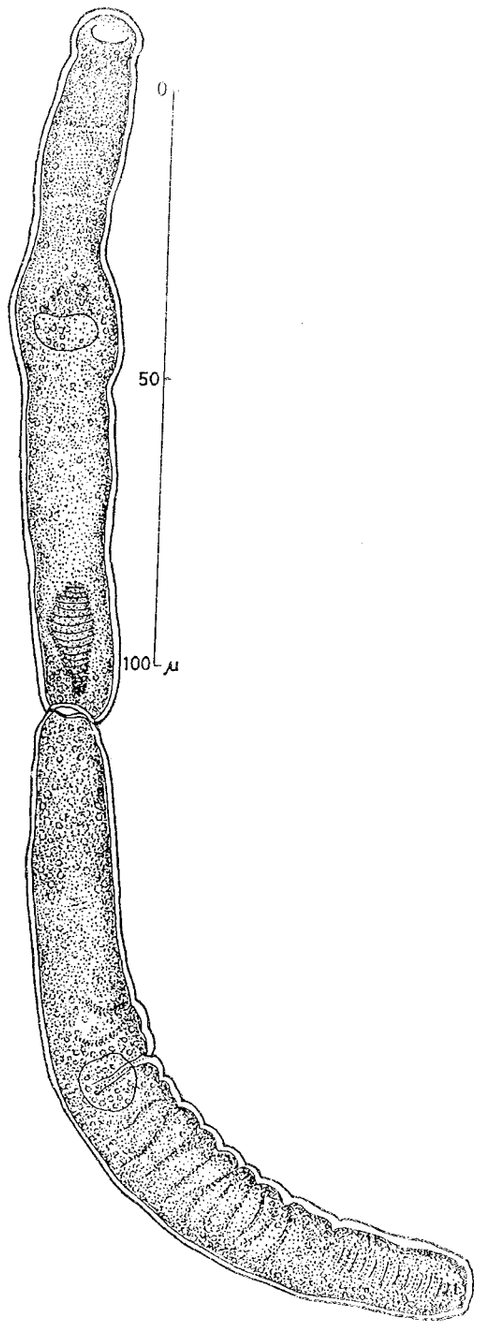

Fig. 7. – *Uradiophora longissima* (Siebold, 1839) Poisson, 1924 - syzygie adulte.

individus pareils comme forme, mais différents comme taille et la densité des inclusions (TL primite = 47 µ, granulations denses; TL satellite = 26 µ, hyalin). Au fur et à mesure que les syzygies continuent leur croissance, celle-ci s'avère inégale comme rythme chez les deux partenaires, car le primite devient beaucoup plus vite volumineux, en se chargeant d'inclusions d'amylopectine, tandis que le satellite peut rester même à l'aspect initial. Les syzygies adultes (LTS = 100-130 µ) (fig.6) sont constituées des gamontes fortement inégaux. Le primite énorme, en forme d'entonnoir, a le protomérite large, ellipsoidal, surmonté à son centre d'un mucron épiméritique conoïde (3,5 µ de hauteur). Son deutomérite conoïdal, aminci caudal, renferme au milieu le noyau et le cytoplasme présente des zones de densité variable par rapport aux enclaves, acquérant un aspect tacheté. Le satellite, d'une forme générale conoïdale peut avoir une taille nettement inférieure et son protomérite est réduit en comparaison avec celui du primite.

Les indices morphométriques des gamontes en syzygies adultes:

	TL	LP	LD	LP:TL	WP	WD	WP:WD	N
primite	93µ	20µ	73µ	1:5	22µ	23µ	1:1	15µ
satell.	30µ	10µ	20µ	1:3	17µ	23µ	1:1,3	8µ

L'espèce est relativement rare (8-10% pourcentage d'infestation) chez des hôtes de Valea Cerbului, où elle s'associe avec *C. rectangularis* et *U. longissima.*

II. Genre *Uradiophora* Mercier, 1912
(Fam. des URADIOPHORIDAE Grassé, 1953)

Eugrégarines parasites des crustacés amphipodes et décapodes, avec des gamontes longs, en file; épimérite simple, convexe, et protomérite réduit, arrondi. Sans stades intracellulaires, les sporozoïtes se développant à la surface de l'épithélium digestif. Sporokystes sphériques, munis d'une crête équatoriale ou des épines radiaires. Syzygies fronto-caudales, à deux ou plusieurs partenaires, parfois ramifiées.

6. *Uradiophora longissima* (Siebold, 1839) Poisson, 1924
Syn: *Gregarina longissima* Siebold, 1839; *Didymophies longissima* Franzius, 1848; *Heliospora longissima* Goodrich, 1949

Céphalins filiformes solitaires, d'âge différent, ayant une taille de 25-160µ, tandis que leur largeur varie seulement entre 5-14µ. Mouvements de glissement et d'infléchissement latéral. Mucron réduit, cylindro-conoïdal (2-5µ hauteur), caduc, saillissant d'une fossette craniale du protomérite arrondi, à aspect d'une couronne (∅ = 8-12µ); à sa base, une bande transversale hyaline s'appuie sur le septum rectiligne qui le sépare de deutomérite étroit, rubané. Celui-ci peut présenter une dilatation au niveau du gros noyau, arrondi-ovalaire, situé au centre, sa région caudale contractile étant tronquée postérieurement. Les syzygies fronto-caudales précoces (gamontes d'env. 60µ de long) continuent leur croissance, jusqu'à une LTS = 200-320µ (fig. 7), les deux partenaires étant à peu près équivalents, disposés parfois en angle obtus.

L'espèce est très répandue. Nous l'avons retrouvée en Roumanie chez *Rivulogammarus* presque dans toutes les stations de récolte, dans un pourcentage supérieur à 50%, mais elle est connue en Europe chez *Gammarus pulex*, *G. roeselii*, *Caprella aequilibra* et *Orchestia littorea* (4) (9). Comme nous, Geus (4) l'a trouvée aussi en Allemagne fréquemment associée avec *C. gammari.*

DISCUSSION ET CONCLUSIONS

Jusqu'à présent il n'y a que le rapport de Ialînskaia (5) qui a signalé chez 2 espèces du s.g. *Rivulogammarus*, c'est-à-dire *G. (R.) balcanicus* et *G. (R.) kischineffensis* du bassin supérieur du Dniestr (Ukraine) des espèces indéterminées des grégarines appartenant aux familles des Cephaloidophoridae et Uradiophoridae. Dans le présent travail, nous faisons connaître pour la première fois chez *Rivulogammarus balcanicus montanus*, caractéristique dans les ruisseaux des Carpates Méridionales en Roumanie, deux espèces, *Cephaloidophora gammari* et *Uradiophora longissima*, à large répartition chez d'autres gammarides, par exemple *G. pulex, G. roeselii, G. fasciatus*. Pour des raisons déjà exposées (1) (2) on garde le rattachement de l'espèce *longissima* de Siebold, 1839 au genre *Uradiophora*, comme nous l'avons fait également pour *Uradiophora ramosa* Bălc-Codr., 1974, parasite chez *Pontogrammarus robustoides* du Delta du Danube. A l'exception de la comparaison des spores, nous signalons des différences discrètes vis-à-vis de la description initiale, completée par Geus (4), mais elles ne justifient pas encore la séparation d' un nouveau taxon.

En ce qui concerne les Grégarines de type trapu et volumineux, parasites des gammarides, il nous semble opportun d'y maintenir le genre *Cephaloidophora* Mavrodiadi, 1908, caractéristique d'ailleurs pour les grégarines des amphipodes. On propose donc le transfert de l'espèce *gammari* de Franzius dans ce genre, sous le nom de *Cephaloidophora gammari* (Franzius, 1848) nov. comb. Nous ne sommes pas d'avis d'encadrer cette espèce dans le genre *Rotundula*, créé ad-hoc par Pixell-Goodrich en 1949, car, dans la diagnose de celui-ci manque un caractère de premier ordre, c'est-à-dire l'existence des stades intracellulaires caractérisant en outre le genre *Cephaloidophora* et déjà connus chez *C. gammari* (6) (7).

En confirmant l'importante diversification morphologique spécifique au sein du genre *Cephaloidophora*, on diagnostique dans ce travail 4 nouvelles espèces établies chez *Rivulogammarus balcanicus montanus*, c'est-à-dire *C. rectangularis* n.sp., *C. crassa* n.sp., *C. dissimilis* n.sp., *C. stentor* n.sp., qui s'ajoutent aux 8 espèces que nous avons décrites antérieurement chez d'autres amphipodes en Roumanie (1) (3).

L'accumulation des connaissances comparées concernant les infestations intestinales grégariniennes chez les gammarides d'éau douce soulève le problème des états polyparasitaires, surtout à l'éventualité des milieux pollués (voire la coexistence d'*Uradiophora longissima* avec des espèces de *Cephaloidophora*, surtout *C. gammari*), ainsi que des aspects de leur biologie cellulaire, rapportés à la croissance cellulaire et aux mécanismes d'attachement des gamontes en syzygies.

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Institut de Biologie
Académie Roumaine

DESCRIPTION OF *BUNAKENIA TANZANIANA* N.SP. (CRUSTACEA, TANAIDACEA) FROM THE EAST AFRICAN MARINE WATERS

MODEST GUȚU

On décrit l'espèce *Bunakenia tanzaniana* n.sp. provenant dans les eaux de la Côte de Tanzanie, de sorte que l'arie de la distribution du genre *Bunakenia* Guțu, 1995 (Famille des Apseudidae Leach, 1814) élargit de Pacifique de sud jusqu'a l'ouest de l'Océan Indien.

Guțu (1995) describes genus *Bunakenia* (with species *B. indonesiana*) from the marine waters near Northern Coast of Sulawesi Island, genus characterized, among others, by the oval-disklike basis of pereopod II and a row of long plumose setae on its antero-sternal margin, correlated with the sexual dimorphism of the chelipeds and merus of pereopods II.

Within the tanaidaceans material (unstudied yet) collected during the Expedition organized by the "Grigore Antipa" Museum of Natural History in 1973 on the Coast of Tanzania, I found some specimens belonging to the above mentioned genus, to a new species whose description is presented further on.

Bunakenia tanzaniana n.sp.
(Figs 1-4)

Material: 5 specimens (2 females, 2 males and 1 juvenile), Mbudia (Tanzanian Coast of the Indian Ocean), 20 m deep, dredging in coarse sand, December 1973.

Description of the female (paratype):

Body (Fig. 1A), robust, flattened dorso-ventrally, of about 3.5 mm length and 0.7 mm width.

Carapace with short, acute, rostrum, and without lateral spine-like apophyses; ocular lobes present, with visual elements.

Pereon has the six free pereonites, wider than long, relatively rectangular, with rounded "corners", without lateral spine-like prolongations. Pereonites three, four and five almost equal and longer than the other ones.

Pleon, formed of five pleonites (short and wide) and pleotelson, has a length equal to that of pereonites two and three or five and six.

Antennule (Fig. 1B) with the first article of basal peduncle massive (almost 2.5 times longer than wide), having simple setae on both sides (inner and outer); the following two articles of the peduncle, relatively short (half from the length of the first article); the second one with long simple setae, on both sides, in the distal end. Inner flagellum has four articles and the outer one, ten (without the common article), the sixth and the eighth article having (besides simple setae) a long aesthetasc.

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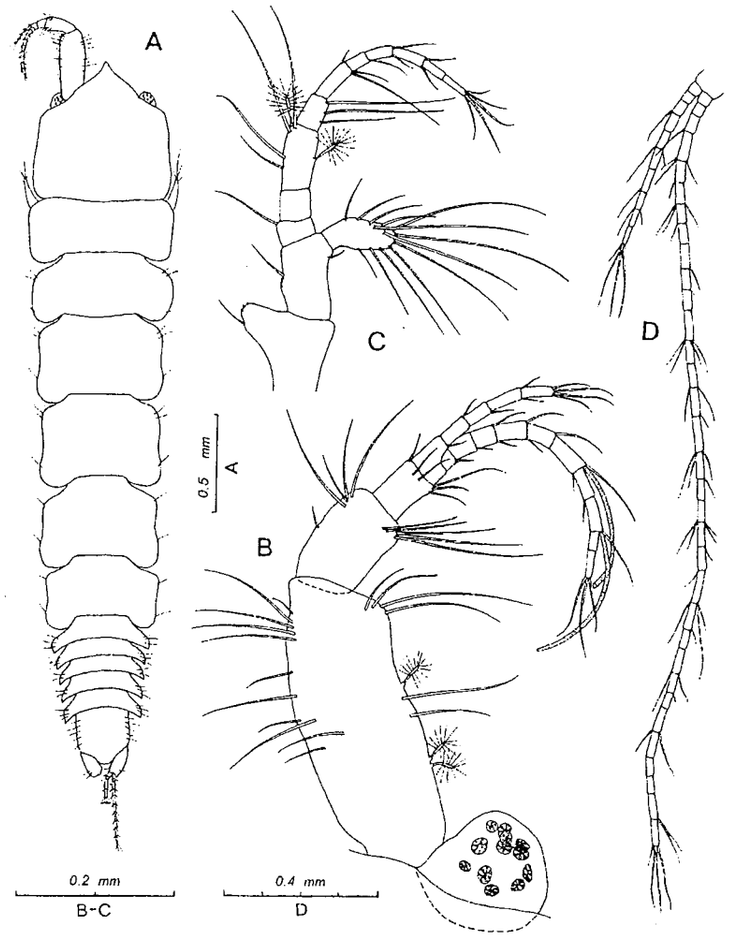

Fig. 1. – *Bunakenia tanzaniana* n.sp., female, paratype: A = body, dorsal view; B = antennule; C = antenna; D = uropod.

Antenna (Fig. 1C) consists of a peduncle formed of five articles and a seven-articulated flagellum, with a few simple setae, longer or shorter. Squama present, well developed, with numerous setae spread around, the apical ones being much longer.

Mandibles (Fig. 2A and B), with palpus, three-articulated, having 9-10 long setae on the first article and 7-8 shorter ones on each of the following two articles. Posteriorly to the palpus, on the mandible's body there is a spine. Pars molaris well developed. Pars incisiva in both mandibles, having four denticles each, and lacinia mobilis of the left mandible with three denticles (Fig. 2B). Setiferous lobes with 6-7 formations without special features.

Labium (Fig. 2C) with oval lobes, having fine hairs on the sides and three apical spines.

Maxillule (Fig. 2D) with two-articulated palpus, ended in five setae, the apical one very long. Inner endite has four apical setae; the outer one with eleven spines on the apical side, and numerous fine long hairs on the lateral margin.

Maxille without special features.

Maxilliped (Fig. 2E and F) relatively small, with a wide and short basis, having in outer "corner" a denticulation, and in the inner one, three spiniform setae. Palpus formed of four articles, the second one as big as the basis. Simple setae of different lengths are distributed on the inner side, as in the drawing. Each endite with seven plumose setae on the inner margin and one-two couplers; apically, with a series of formations of different forms and sizes; caudo-distal seta simple and long (Fig. 1F).

Epignath (Fig. 2G) big, scoop-like form, with a long spine.

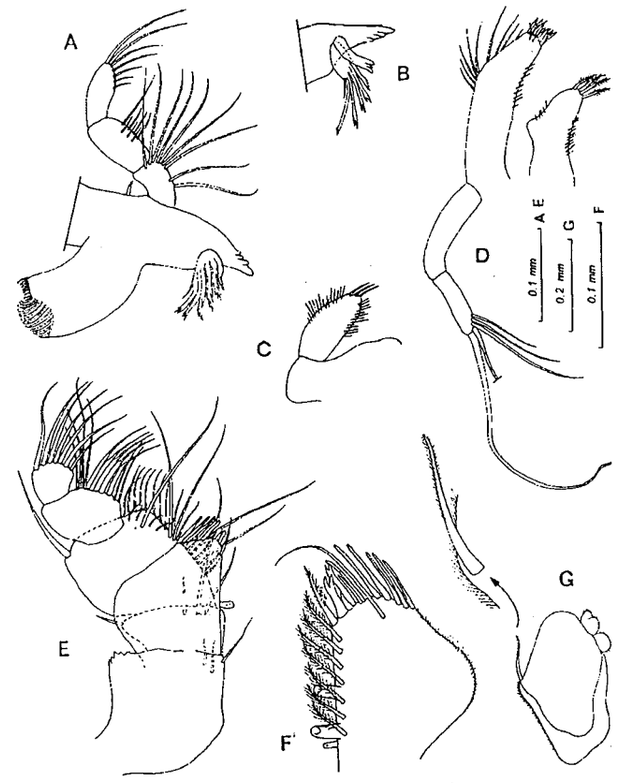

Fig. 2. – *Bunakenia tanzaniana* n.sp., female, paratype: A = right mandible; B = distal part of left mandible; C = labium, right part; D = maxillule; E = maxilliped; F = endite of the symmetrical maxilliped; G = epignath.

Cheliped (Fig. 3A) with very small coxa. Basis has a strong spine and three plumose setae on the postero-tergal margin; exopodite present, well developed. Merus relatively small, with 11-12 simple, long, setae. Carpus narrow and long, also with many setae on the sternal side. Propodus shorter than carpus, finger being almost half of its length; claw very small. Dactylus thiner than the finger of the propodus and a strong claw.

Pereopod II (Fig. 3B) much bigger and stronger than the cheliped or the following pereopods. Coxa with a strong spine, ended in a long plumose seta. Basis relatively short, but wider, has a row of long plumose setae on the antero-

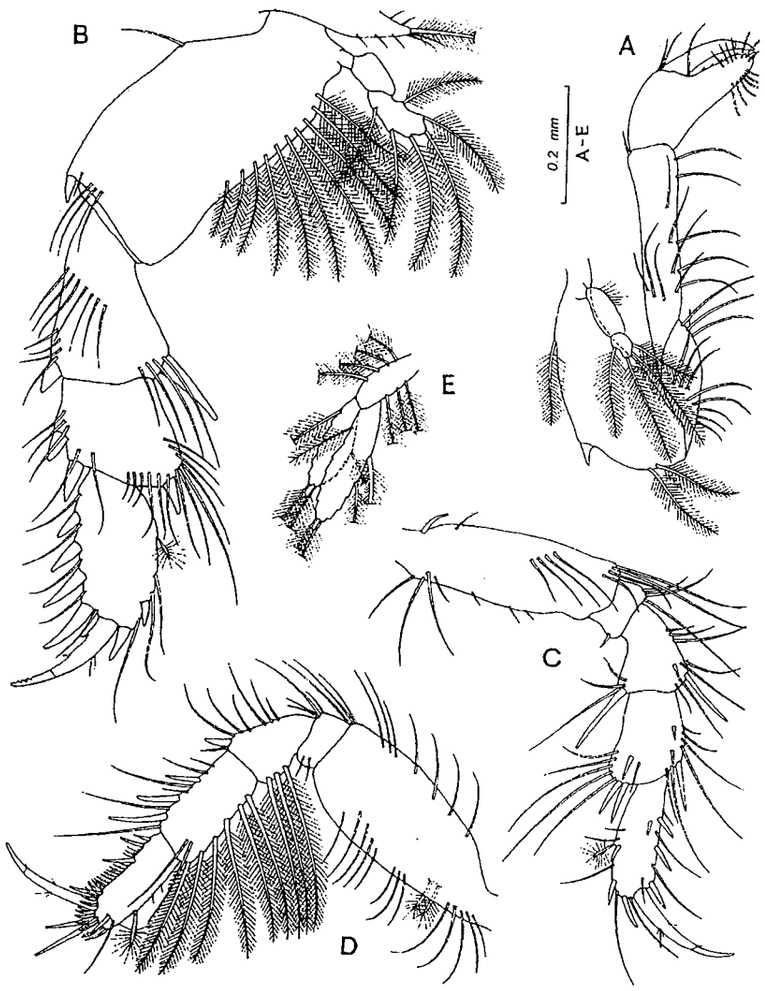

Fig. 3. – *Bunakenia tanzaniana* n.sp., female, paratype: A = cheliped; B = pereopod II; C = pereopod III; D = pereopod VII; E = pleopod.

sternal margin; exopodite present, well developed. Ischium very short. Merus distally widened, with a spine on each margin and some simple setae; a row of seven simple setae in a diagonal line on the outer surface. Carpus short and wide, with two spines on the sternal margin and a tergal-terminal one bordered by long and short setae. Propodus narrower and longer than carpus, has four strong spines on the sternal side and two spines behind dactylus inserted with a thin seta each, those from the tergal margin being longer. Dactylus relatively thin, with two little spines on the inner margin; claw thin, dented on the inner curve.

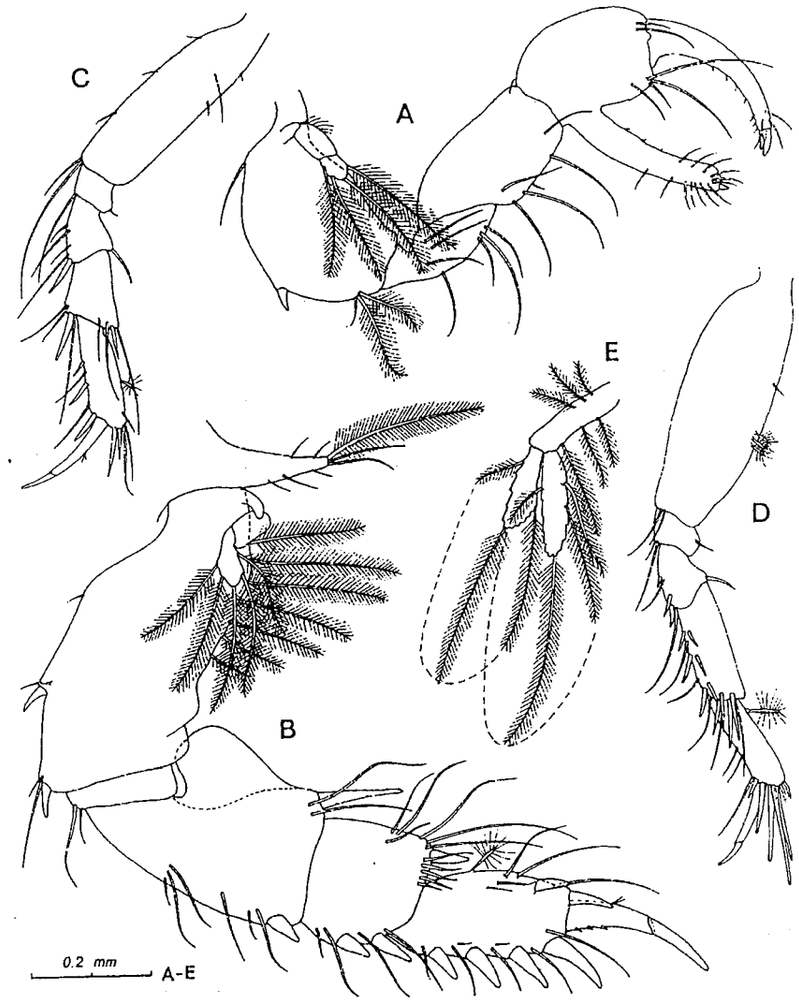

Fig. 4. – *Bunakenia tanzaniana* n.sp., male, paratype: A = cheliped; B = pereopod II; C = pereopod IV; D = pereopod V; E = pleopod.

Pereopod III (Fig. 3C) much smaller than the previous one, with a thin basis, having a spine at the proximal end of the sternal side, and several setae. Ischium, merus, carpus and propodus with fine spines of different lengths and several setae as in the drawing. Dactylus long, with the claw slightly curved.

Pereopods IV-VI broken.

Pereopod VII (Fig. 3D) with the basis slightly swollen in comparison with that of the pereopod III and with fine setae on sides. Merus and carpus with three, respectively, six long plumose setae on the tergal margin; on the sternal side there are simple setae and one-three spines. Propodus with a row of ciliated setae on the sternal and apical margins, among them long, thin spines being remarked. Dactylus long, with the claw approximately short.

Pleopods (Fig. 3E) biramous, in five pairs well developed. The endopodite longer than the exopodite, both rami having around them very long plumose setae.

Uropods (Fig. 1D) filiform; the endopodite long, formed of about 33 articles and the exopodite of ten.

Males (Fig. 4A-E), resembling very much with the females, excepting chelipeds and pereopods II. Chelipeds (Fig. 4A) have the basis, merus and carpus stronger than in females, the most important difference being remarked at the level of the fingers of the propodus and of the dactylus, which are long and thin, curved like sickle. Pereopod II (Fig. 4B) is larger than in females and has less setae on the antero-sternal margin of the basis; the most obvious dimorphic feature is in merus which has a tergal lamellar prolongation at the ischium joint. At the leg flexion this prolongation "couples" with the basis increasing the resistance of the pereopod. Pereopods IV and V as in the figure 4C and D. Pleopods (Fig. 4E) resemble with those of the females, the setae being slightly shorter.

Remarks: Because the pereopods IV-VI from the female and VI from the males were broken, in the dissected specimens, I could not make their description. Studying pereopods III and VII present in both sexes, I observed that they are practically identical, leading me to the idea that also the other pereopods of both sexes resemble. That is why I illustrated pereopods IV and V of males (pereopod VI missing, as I have already mentioned), illustrations which can help for identification of the females. But it has to be pointed out that pereopod IV is slightly smaller than the previous one and V-VII have the basis slightly swollen than in pereopods III and IV, in both sexes.

Type locality: Mbudia (Tanzanian Coast), the Indian Ocean.

Etymology: the name of the species was given according to the name of the country from where it was collected, *Tanzania*.

Holotype: female with marsupium, preserved in the Collections of "Grigore Antipa" Museum of Natural History of Bucharest, having No. 250,113.

Allotype: adult male, in the same collection, with No. 250,114.

Paratypes: 3 specimens (1 subadult female, 1 adult male – dissected specimens – and 1 juvenile), in the same collection, having No. 250,115.

Remarks: In comparison with *Bunakenia indonesiana* Guțu, 1995, the new species (*B. tanzaniana*) distinguishes by the number of the articles of the antennule and antenna, basis form of the pereopods II and by the number of the setae on its antero-sternal margin.

From the ecological point of view, both species of the genus *Bunakenia* Guțu, 1995 were collected from the shallow marine bottom, with coarse sediments near the coral reefs.

By the description of this species the distribution area of genus *Bunakenia* enlarges, from southern Pacific to the west of the Indian Ocean.

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HISTOCHEMICAL LOCALIZATION OF MERCURY IN THE OVARY OF CRUCIAN CARP, *CARASSIUS AURATUS GIBELIO*, AFTER EXPOSURE TO MERCURIC CHLORIDE

RADU MEŞTER, OTILIA ZĂRNESCU

The deposition of mercury in the ovary was studied in the crucian carp, *Carassius auratus gibelio*. The fish were exposed to sublethal concentrations of mercuric chloride($HgCl_2$) in two experimental variants:
1. Injected intraperitoneally (500 μg) and sampled after 2, 3, 7, 24 hours and 2, 4, 7 days;
2. Dissolved in the aquarium water (0,1 and 1 μg/l) for 24 hour waterborne and 8 day exposures.
Ovaries were fixed and sections were developed by autometallography, a method whereby accumulations of mercury sulfides are silver amplified.
In the previtellogenic follicles accumulation of mercury was observed in the cortical cytoplasm and within cortical alveoli.
In the vitellogenic follicles silver-enhanced mercury deposits were predominantly localized in: zona radiata, periphery of cortical alveoli and yolk platelets.

Heavy metal pollution in the aquatic environment is a major problem because of the toxicity of these metals and their persistence and tendency to accumulate in organisms.

Fish accumulate mercury as a result of an efficient uptake and a low rate of elimination (Olson et al., 1973). The concentration of mercury retained by a specific tissue depends on the metal concentration in water or food, time of exposure and rate of metabolism or elimination from the tissue (McKim et al., 1976; Olson et al., 1978).

Mercury is very toxic in fish and induces morphological and physiological alterations (Hawryshyn et al., 1982; Menezes and Qassim, 1984).

The oocytes of most nonmammalian vertebrates owe their large size primarily to the receptor-mediated endocytosis of yolk protein precursors vitellogenin (VTG), made by the liver (Opresko et al., 1980). After internalization VTG is cleaved into several smaller fragments including: lipovitellin, phosvitin and phosvette (Lange, 1985). In these forms, the proteins are stored for prolonged periods as lipoprotein crystals within membrane-bound yolk platelets.

Several xenobiotic compounds affect serum levels of vitellogenin, the growth of oocytes and the deposition of the yolk, in different fish species (Lesniak and Ruby, 1982; Chen and Sonstegard, 1984; Haux et al., 1988; Ruby et al., 1993; Pereira et al., 1993).

Accumulation of mercury in fish was studied by quantitative methods which gave information on retention and elimination of the metal from different organs of fish under various experimental conditions. In the past studies of the uptake of mercury by the fish ovary (Lockhart et al., 1972; Weisbart, 1973; McKim et al.,

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1976; Olson et al., 1978) we did not find any indication about the histochemical localization of this metal in the ovary of crucian carp, *Carassius auratus gibelio*. The histochemical method of autometallography (AMG) is the most sensitive method for detecting mercury within an organ and its cells (Danscher, 1984; Danscher and Møller-Madsen, 1985). This technique is based on the ability of mercury sulphide and mercury selenide accumulations to catalyze the reduction of silver ions to metallic silver in the presence of an electron donor. Metallic silver forms a shell (silver enhancement) around the mercury compounds in the tissue (Danscher 1991).

In the present study we used the autometallographic technique to detect mercury deposits in the ovary of *Carassius auratus gibelio*, exposed to sublethal concentrations of mercuric chloride ($HgCl_2$) administered in two routes of exposure: intraperitoneal and dissolved in aquarium water.

MATERIALS AND METHODS

Female crucian carp, weighing between 30–40 g were purchased from the Fisheries research Farm-Nucet. The specimens were acclimatized to laboratory conditions for two weeks, at room temperature in glass aquaria, containing tap water (500 l).

All observations were made in June-July, 1994.

MERCURY EXPOSURE

The following experiments were carried out to evaluate the effect of mercury ($HgCl_2$ of analytical grade, purchased from Merck, Germany) on the fish ovary:

1. *Intraperitoneal administration.* Each fish was injected intraperitoneally (IP) with 500 μg $HgCl_2$ dissolved in a saline solution isotonic for freshwater teleost. After injections the fish were quickly returned to glass aquaria (30 l) and 21 fish were sacrificed at 2, 3, 7 and 24 hours and 2, 4 and 7 days after injections.

2. *Aquarium administration.* $HgCl_2$ was added to aquarium water (30 l) as a mercuric chloride solution at the beginning of the experiments, one hour before addition of fish. The concentrations of mercury used in this study were 1 and 0.1 μg/l. The animals were sacrificed after 24 hours or 8 days.

There was no mortality as a result of the exposure to mercuric chloride. Five fish were kept in clean freshwater, serving as controls.

HISTOLOGICAL PROCEDURES

After mercury treatments, the fish were sacrificed by medullary transection. Small pieces of tissue were dissected from the central part of the ovaries, immersed in Bouin's solution or in 10% phosphate buffered formalin pH–7.2, with 2% DMSO (overnight), dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections (5 μ) were silver-enhanced for microscopic analysis following the autometallographic method of Danscher (1984) and Danscher and Møller-Madsen (1985). Accumulations of mercury bound to sulfide act as catalysts for the

transformation of adhering silver ions into metallic silver (Timm et al., 1966; Danscher and Møller-Madsen, 1985). After the transformation, the silver atoms are catalytic themselves and the original catalysts become encapsulated by layers of silver. Following physical development, the sections were stained with alcian blue or 1% neutral red.

RESULTS

After the autometallographic development, the mercury deposits (made visible by the silver grains) were seen only in the sections from fish exposed to mercury and not in the control (non-exposed) animals.

The ovary of fish used in the first type of experiment was characterized by the presence of early and late previtellogenic follicles with cortical alveoli. Cortical alveoli make their first appearance in the peripheral ooplasm. By the end of previtellogenic stage, cortical alveoli fill almost the entire oocyte cytoplasm, but in subsequent stages they continue to form and are displaced at the periphery of the oocyte by yolk proteins which accumulate centripetally. In the oocyte of crucian carp, the cortical alveoli consist of colloidal material, a spherical body and often a membraneous structure.

The chorion (zona radiata) appears coincident with cortical alveoli and is first observed as a thin band between the oocyte and the overlying follicle cells.

In the ovary of fish IP injected with 500 μg $HgCl_2$, at two hours after exposure to mercury the silver-enhanced mercury deposits were localized in the previtellogenic follicles as a dark layer at the chorion level (Fig. 1). A small number of early and late previtellogenic oocytes show silver grains in the cortical cytoplasm.

At three hours after the injection, deposits of mercury are present in the cortical cytoplasm of late previtellogenic oocytes (Fig. 2). At this time it is possible to detect the beginning of the mercury accumulation in the central spherical body of the cortical alveoli (Fig.3). The highest density of silver-enhanced mercury was found at four and seven days after injection, in late previtellogenic oocytes (Fig. 4) within the central spherical body of cortical alveoli (Fig. 5).

The ovary of fish from the second type of experiment is characterized by the presence of a large number of vitellogenic follicles. These oocytes can be recognized by the presence of yolk platelets which fill the whole cytoplasm. The cortical alveoli are larger, completely formed and uniformly distributed around the oocyte periphery beneath the oolema. The zona radiata is very thick and shows pore canals.

Twenty four hours after metal exposure, silver-enhanced mercury was observed in the pore canals of the zona radiata (Fig. 6). Also in this type of experiment mercury was accumulated in the cortical alveoli, but in this case in the colloidal material not in the central body (Fig. 7). The beginning of the accumulation of mercury on the surface of yolk platelets (Fig. 8) was observed at this time. This accumulation became much stronger eight days after exposure when the silver grains were present as longitudinal layers (Fig. 9).

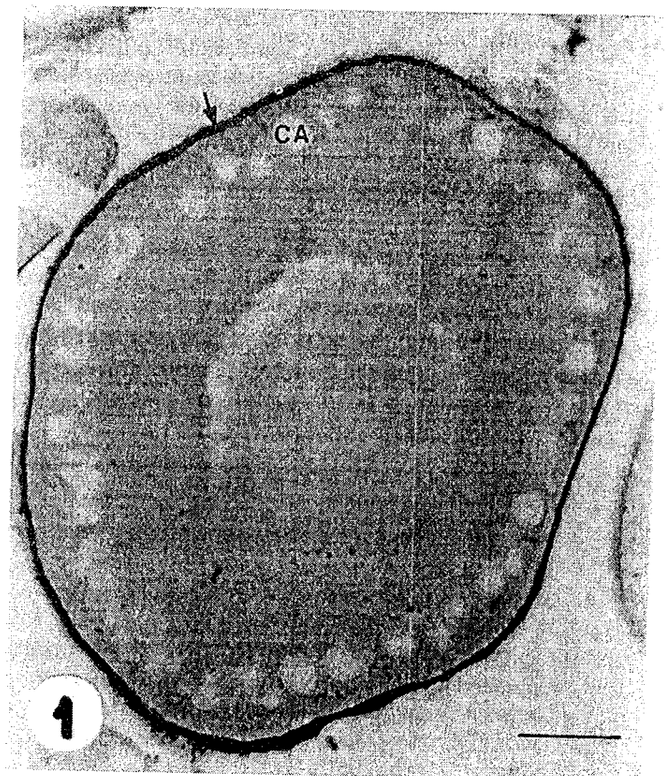


Fig. 1. – Previtellogenic follicle with cortical alveoli (CA). At 2h after IP injection. Silver-enhanced mercury deposits appear as a localized, dark layer at the chorion level (arrow). Scale bar = 100 μm.

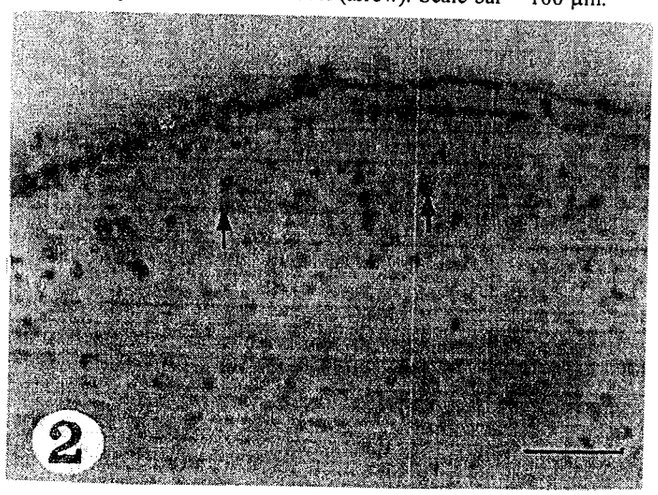
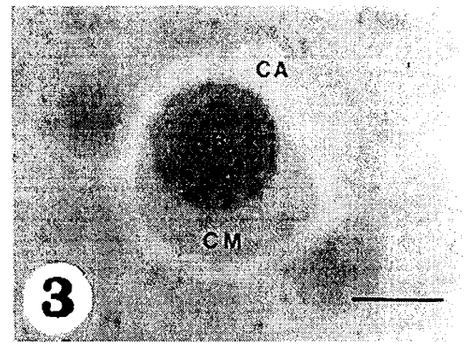


Fig. 2. – Mercury deposits localized within cortical cytoplasm (arrows) in early previtellogenic oocytes (3h after IP injection). Scale bar = 10 μm.

Fig. 3. – At 3 h after IP injection it is possible to detect the beginning of the mercury accumulation in the central spherical body (SB) of the cortical alveoli (CA). Colloidal material (CM). Scale bar =10 μm.



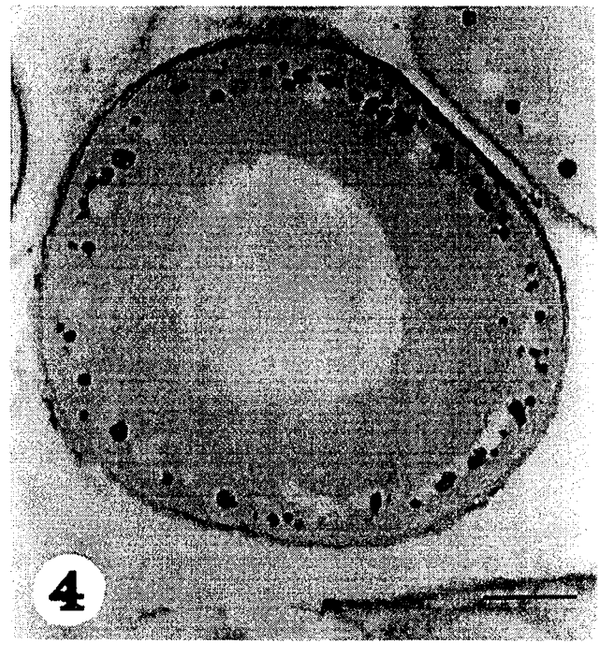


Fig. 4. – The late previtellogenic oocyte showed the highest content of mercury in their cortical cytoplasm, at 4 days after IP injection. Scale bar = 100 μm.

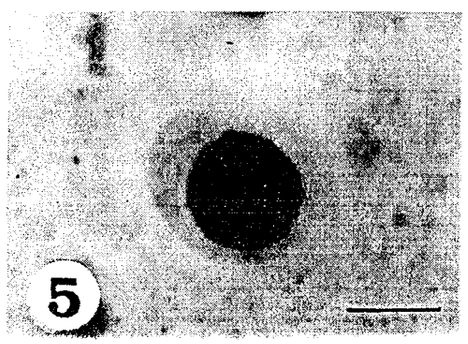


Fig. 5. – At 7 days after IP injection, cortical alveoli contained highest content of mercury in their spherical body.

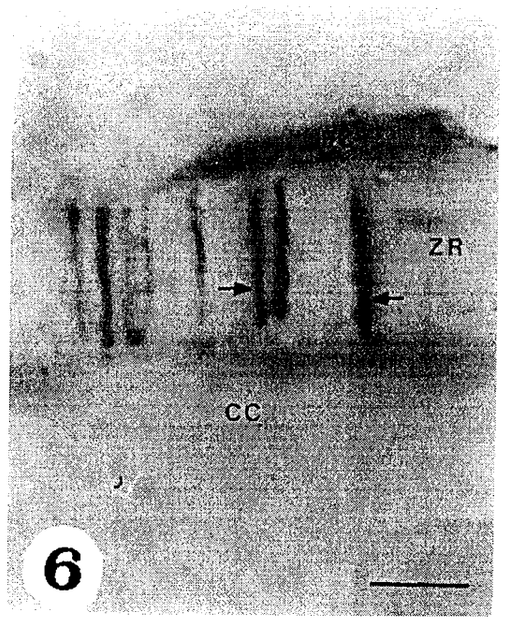
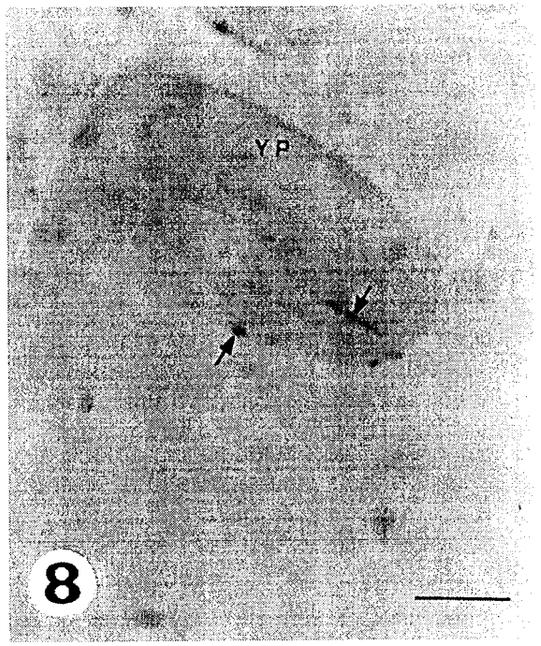


Fig. 6. – After 24 h from exposure to metal (dissolved in aquarium water) silver–enhanced mercury was observed in the pore canals of zona radiata (ZR) (arrows). Follicle cell (FC); Cortical cytoplasm of oocyte (CC). Scale bar = 10 μm.

Fig. 8. – At 24 h after metal exposure it was also observed the beginning of the accumulation of mercury on the surface of yolk platelets (YP)(arrows). Scale bar = 10 μm.



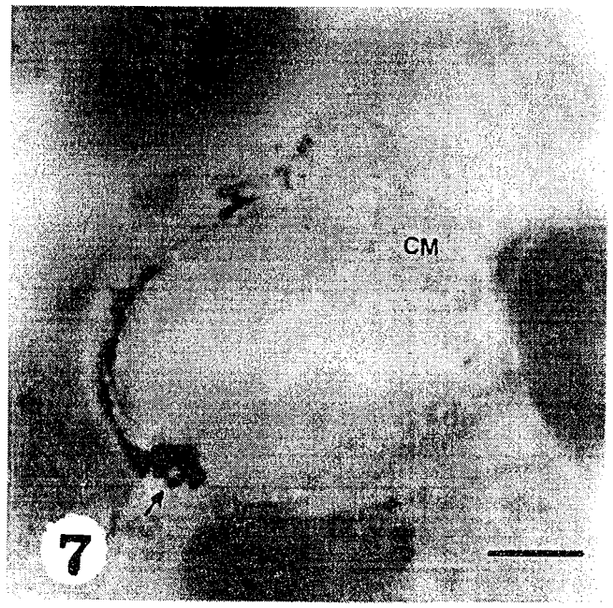


Fig. 7. – In the cortical alveoli of vitellogenic oocyte accumulation of mercury was observed within colloidal material (CM) at 24 h after metal exposure in aquarium water(arrow). Scale bar = 10 μm.

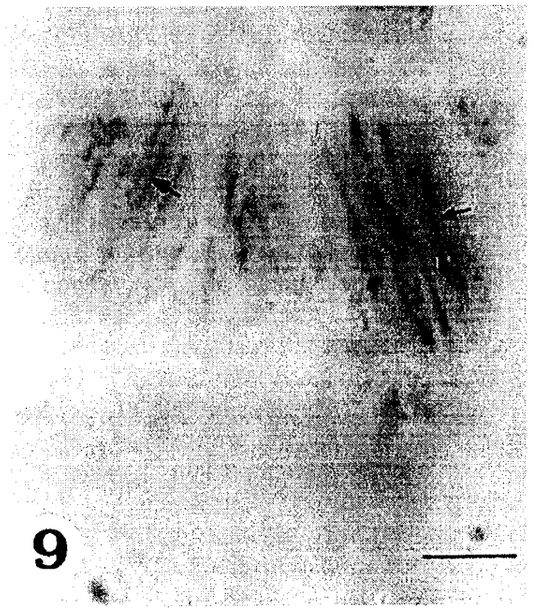


Fig. 9. – The accumulation of silver grains becomes much stronger at 8 days after metal exposure (aquarium water), when mercury deposits were present as longitudinal layers (arrow). Scale bar = 10 μm.

DISCUSSION

In the present study, accumulation of mercury has been demonstrated in: the zona radiata, cortical alveoli and yolk platelets of the oocytes of crucian carp, exposed to mercuric chloride.

Mercury begins to accumulate in the cytoplasm of previtellogenic oocytes approximately three hours after IP injection. In a quantitative study (Weisbart, 1973) also reported that the ovary of goldfish manifested the highest levels of $^{203}Hg(NO_3)_2$ at three hours and eight days after IP injections. The cortical alveoli of some teleosts consist of a colloidal material, a spherical body and often a membraneous structure (Iwamatsu and Ohta, 1976; Iwamatsu and Keino, 1978). The cortical alveoli in the oocytes of some fresh-water teleosts are composed of a complex polysaccharide-protein (Donato et al., 1980); their protein content is rich in cysteine and tyrosine whereas the polysaccharide content consists of chondroitin sulphate-β and traces of polygalacturonic acid. It has been established that mercury bound to endogenous sulfide serve as catalytic centers for the reduction of silver ions to metallic silver (Timm et al., 1966; Danscher and Møller-Madsen, 1985).

In the previtellogenic oocytes mercury was preferentially accumulated in the central spherical body of cortical alveoli while in the vitellogenic oocytes where these structures are entirely formed, the deposits of mercury were mainly accumulated at their periphery, in colloidal material. This differential accumulation may be a result of differential uptake of mercury in the two stages of oocyte development, or it may be a consequence of the method by which mercury was administered (IP or dissolved in the aquarium water). Accumulation of metals (gold) was also demonstrated, ultrastructurally, in the cortical alveoli from ovaries of rats injected IP with sodium aurothiomalate (Møller-Madsen et al., 1985).

Quantitative studies have shown that inorganic mercury is concentrated in the gonad and eggs to levels which substantially impaired development and survival of embryos and alevins maintained in mercury-free control water (Birge et al., 1979). Results from various crosses between control and experimental populations exposed to mercury further indicated that eggs and sperm were both affected by the chronic exposure of the adult prior to spawning (Birge et al., 1979). Analyses at hatch of both the yolk and embryo indicated approximately 50% of the initial organic mercury was contained in the yolk of brook trout, *Salvelinus fontinalis* (McKim et al., 1976).

Vitellogenin is the principal precursor to yolk protein in oviparous vertebrates (Wallace 1985). This lipoglycophosphoprotein is produced in the liver and released into the blood-stream, sequestered by developing oocytes and proteolytically cleaved into smaller proteins such as lipovitellin, phosvitin and phosvette (Lange 1985). The proteolytic products are stored as yolk (Wallace and Jared 1969). Interference in the production of vitellogenin in the liver, or its subsequent uptake by the gonad, would affect egg production and fecundity.

Various xenobiotic compounds affected serum VTG levels. Cadmium (Haux et al., 1988) and Polychlorinated biphenyls (PCB) (Chen and Sonstegard 1984)

both lowered blood VTG levels in salmonids. Exposure to cyanide during vitellogenesis in salmonids produces variable results depending on the timing of the exposure. Exposure during early vitellogenesis lowered VTG levels in the blood perhaps by interfering with VTG production as suggested by Ruby et al. (1986). Exposure later in the reproductive cycle elevated VTG through blocking its uptake by the gonad (Ruby et al., 1987). Lesniak and Ruby (1982) reported that cyanide exposure altered the pattern of yolk deposition and increased rates of oocyte atresia in rainbow trout, *Oncorhynchus mykiss*. Other investigators have reported that elevated cadmium levels in the liver were associated with lower serum VTG levels and reduced gonadosomatic indices (Pereira et al. 1993).

Exposure to Hexachlorocyclohexane (β-HCN) induces excessive vitellogenesis and yolk formation in premature oocytes from juvenile female guppies, *Poecilia reticulata* while methyl mercury does not seem to affect the female gonads (Wester 1991).

In the sockeye and coho salmon, *Oncorhynchus nerka* and *kisutch*, inorqanic mercury ($HgCl_2$) or methylmercury (CH_3HgCl) are incorporated in lipoproteins (Reichert and Malins, 1974) and their interaction with salts of heavy metals (Hg, Cd) changes the surface structure of the lipoprotein (Reichert and Malins, 1975).

Our microscopic findings demonstrated that mercury was accumulated within the yolk platelets. Several explanations may account for these results. First, mercury may bind to VTG and in this form is incorporated into the ovary. Second, mercury may combine inside the oocyte with the products of proteolytic cleavage of VTG. Third, inorganic mercury may bind directly to yolk platelets already formed.

In the amphibians mercury impairs the development of primordial germ cells, formation of the first growth phase of oocytes, the growth and maintenance of second growth phase oocytes and the cleavage processes and embryonic development (Byrne et al., 1975; Power et al., 1989; Punzo 1993).

Also, exposure to mercuric chloride impairs the estrous cycle as well as the growth of ovarian follicles in mammals by reducing the secretion of pituitary gonadotropins and ovarian steroids (Lamperti and Niewenhuis, 1976; Clarkson et al., 1983)

The accumulation of mercury in the female ovary was also detected by the autometallographic technique in the bivalve molluscs, *Anodonta piscinalis* in which the silver-enhanced mercury was localized in the cortical cytoplasm of vitellogenic oocytes, follicle cells and in the oocyte nucleolus (Zărnescu et al., 1994).

In fish, accumulation of mercury has been demonstrated to occur in lysosomes and extracellularly in the basal lamina of proximal tubules of the kidney and in the nucleus, endoplasmic reticulum and lysosomes of the hepatocytes of the liver (Baatrup et al., 1986).

In conclusion, to our knowledge this is the first report about localization in the ovary of crucian carp of inorganic mercury detected by autometallographic technique. This study has revealed some sites of mercury accumulation in the ovary such as: zona radiata, cortical alveoli and yolk platelets.

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EFFECTS OF MERCURIC CHLORIDE EXPOSURE IN THE FOOT OF *ANODONTA PISCINALIS* (NILSSON). HISTOPATHOLOGICAL AND HISTOCHEMICAL FEATURES

OTILIA ZĂRNESCU, VIORICA MANOLACHE, CĂLIN TESIO

The histopathological changes induced by mercury and localization of this metal in the foot was studied in the fresh water mussel, *Anodonta piscinalis*.
The mussels were exposed to sublethal concentrations of mercuric chloride ($HgCl_2$) in the following experimental variants:
1. 5 μg/1 $HgCl_2$– 4 days; 2. 5 and 10 μg/1 $HgCl_2$ – 24 hours; 3. 5 and 10 μg/1 $HgCl_2$ – a month; 4. 0,1 and 1 μg/1 $HgCl_2$ – 4 months.
Numerous aspects of histopathological changes were noticed, especially, disruption of the surface epithelium and connective tissue architecture between muscle bundles, abnormal concentration of hemocytes in the outermost zone of the foot and atrophy of muscle bundles.
Autometallographically silver-enhanced mercury deposits were localized by light microscopy in: apical pole of epithelial cells, on the basal surface of the epithelium, in the connective tissue around muscle bundles, between muscle fibbers, at the basis of hemolymph vessels and in the hemocytes.

INTRODUCTION

Bivalve molluscs both fresh water and marine have been shown to be effective concentrators of trace elements and have proved useful as indicators of heavy metals pollutions (10).

It is known that mussels are good accumulators for a number of heavy metals (12). Since they are characterized by a filter feeding behaviour, when they pump the water through the gills and take up large amounts of small particles as food, they ingest various substances dissolved in the water or incorporated into algae, bacteria and other particulate substances. Salánki and V-Balogh (13) found that the fresh water mussel, *Anodonta cygnea*, similar to the other molluscan species, accumulates high amounts of heavy metals and its concentration capacity may reach a several thousands value in various organs as compared to the concentration of metal in the surrounding water. The concentration capacity of various organs is different, the highest amount of accumulated metals was detected in the kidney and gills while the lowest levels were detected in the adductors (14, 16).

Accumulation of heavy metals in the foot of bivalve molluscs was studied mainly by quantitative methods (3, 4, 9, 11, 13, 16) which gave information on retention and elimination of the metal under various experimental conditions. In the past studies of the uptake of metals by the bivalve (3, 4, 8, 9, 11, 13, 16) we did not find any indication about histochemical localization of mercury in the foot of these invertebrates.

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The histochemical method of autometallography (AMG) is the most sensitive method for detecting mercury within an organ and its cells (5, 6, 7). This technique is based on the ability of mercury sulphide and mercury selenide accumulations to catalyze the reduction of silver ions to metallic silver in the presence of an electron donor. Metallic silver forms a shell (silver enhancement) around the mercury compounds in the tissue (5).

In the present study we described the histopathological alteration and used the AMG technique to detect mercury deposits in the foot of *Anodonta piscinalis* exposed to sublethal concentrations of mercuric chloride.

MATERIALS AND METHODS

The specimens of *Anodonta piscinalis* (Nilsson) were acclimatized to laboratory conditions (for 7 days and 4 months), at room temperature in glass aquaria containing tap water (500 1). The mussels were exposed to sublethal concentrations of mercury ($HgCl_2$ of analytical grade, purchased from Merck, Germany) in the following experimental variants: 1.5 µg/1 $HgCl_2$ - 4 days; 2.5 and 10 µg/1 $HgCl_2$ - 24 hours; 3.5 and 10 µg/1 $HgCl_2$ - a month; 4. 0,1 and 1 µg/1 $HgCl_2$ - 4 months.

At the beginning of the experiments, and one hour before the addition of mussels, $HgCl_2$ was added to aquarium water (30 l) as a mercuric chloride solution. There was no mortality as a result of the exposure to mercuric chloride. After mercury treatments the small pieces of ventral region of the foot were dissected, immersed in Bouin's solution or in 10% phosphate buffered formalin-pH = 7.2 (overnight), dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections(5 µ) were stained with haematoxylin-eosin. Mercury accumulations in the foot were silver-enhanced for microscopic analysis according to the AMG method of Danscher (5) and Danscher and Møller-Madsen (6). Treatment of the sections with 0.1 N HCl and 1% KCN did not dissolve the catalyst, demonstrating that the catalyst could be neither zinc nor copper sulfide. Following physical development, the sections were stained with 1% neutral red.

RESULTS

The foot is one of the characteristic organs of molluscs. It is situated on the ventral side of the body under the visceral mass. It is a muscular organ differentiated from the musculature of the ventral wall of the body. The great mobility of the foot is given by the presence of two systems of muscle. From outside to inside there were identified four superposed muscle layers separated by connective tissue. At the foot surface there is a simple ciliated columnar epithelium (Fig. 1). The underlying muscle and connective tissue are arranged in a regular pattern.

Intoxication of *Anodonta piscinalis* with $HgCl_2$ induces histopathological changes characteristic for each experimental variant. Following AMG development, deposits of silver grains were observed only in the sections from mussels exposed to mercury and not in the control (non-exposed) animals.

Fig. 1. – The simple ciliated columnar epithelium of the foot. Scale bar = 25 µm.

In the presence of *5 µg $HgCl_2$ - 24 hours* on certain portions it is observed the loss of cilia from epithelial cells, and disruption of the connective tissue architecture between muscle bundles (Fig. 2). At the same period of time, but at a higher dose

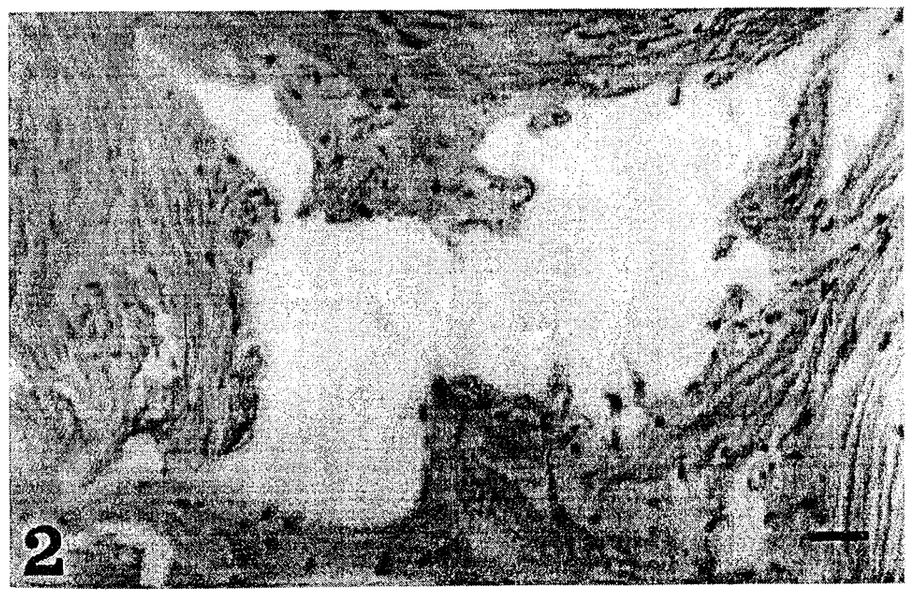

Fig. 2. – Disruption of the connective tissue architecture between muscle bundles at 24 h after mercury exposure - 5 µg/l $HgCl_2$. Scale bar = 50 µm.

Fig. 3. – After 24 h from exposure to 10 µg/l $HgCl_2$ the foot tip exhibiting loss of outer epithelium. Scale bar = 50 µm.

(*10 µg/l $HgCl_2$*) the ventral epithelium of the foot was disrupted to some degree (Fig. 3), epithelial cell nuclei being pyknotic. At the both doses by the AMG method we identified the presence of mercury on the surface and in the apical pole of epithelial cells (Fig. 4). In the apical region of the epithelium, mercury deposits were displayed

Fig. 4. – Deposits of silver-enhanced mercury were observed on the surface epithelium. In the apical portions of epithelial cells and in the underlying connective tissue the mercury deposits appeared in the form of black inclusions (arrows). 24 h after exposure to 10 µg/l $HgCl_2$. Scale bar = 50 µm.

Fig. 5. – Mercury deposits observed between muscle fibers (arrows). Mussels intoxicated with 5 µg/l $HgCl_2$ - 24 h. Scale bar = 50 µm.

in the form of black bodies, probably lysosomes. Mercury accumulation is low in the connective tissue and between muscle fibers (Fig. 5).

After *a month* from metal exposure (*5 and 10 µg/l $HgCl_2$*) the surface epithelium is destroyed with vacuolated epithelial cells and without cilia (Fig. 6).

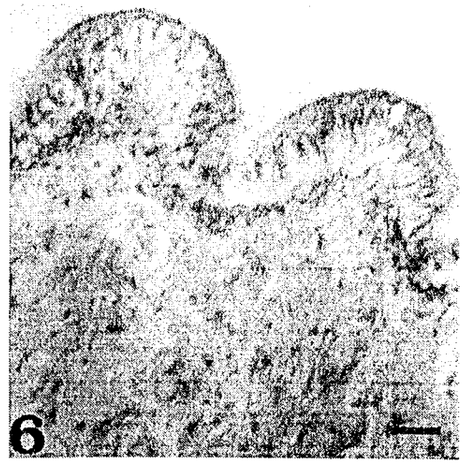

Fig. 6. – After a month from mercury exposure (5µg/l $HgCl_2$) the surface epithelium is destroyed with vacuolated cells. Scale bar = 50 µm.

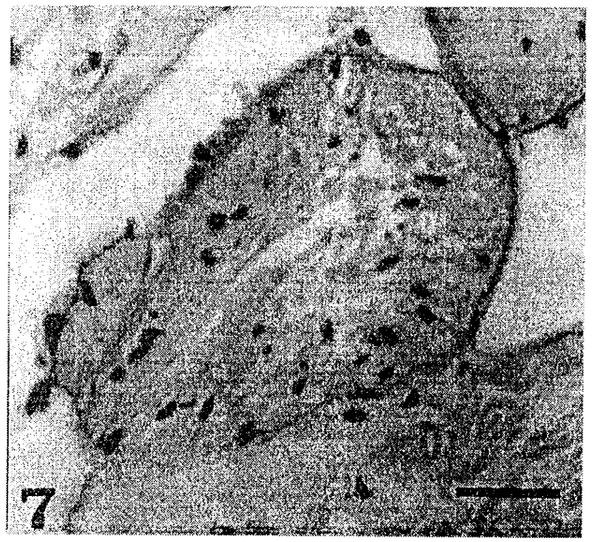

Fig. 7. – Degenerated muscular bundles surrounded by a thin connective layer. Mussel intoxicated with 10 µg/l $HgCl_2$ a month. Scale bar = 50 µm.

Some nuclei of muscle fibers and those of connective cells are pyknotic. Abnormal concentrations of hemocytes were seen in the outermost zone of the foot and around hemolymph vessels. Among normal muscle bundles appear degenerated muscular masses surrounded by a thin connective layer (Fig. 7). The silver-enhanced mercury deposits were localized in the apical pole of epithelial cells and in the hemocytes from connective tissue (Fig. 8).

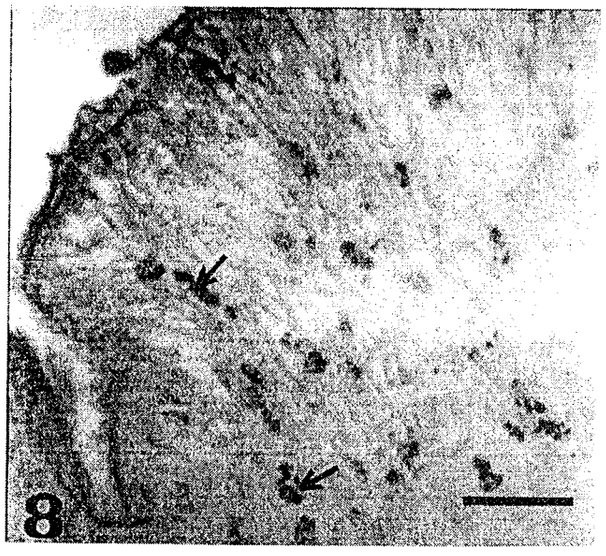

Fig. 8. – The silver-enhanced mercury deposits were localized in the apical pole of epithelial cells and in the hemocytes from connective tissue (arrows). 5 µg/l $HgCl_2$ - a month. Scale bar = 50 µm.

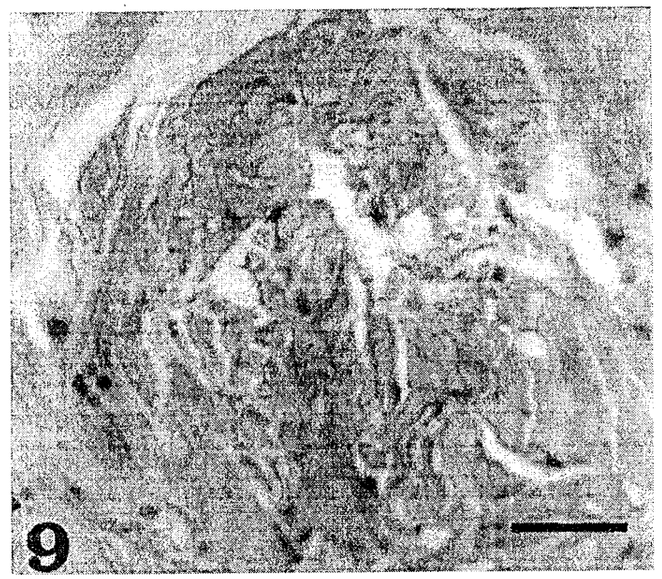

Fig. 9. – Atrophy of muscle bundles at four months after mercury exposure (0.1 µg/l $HgCl_2$). Scale bar = 50 µm.

In the third experimental variant (intoxication with *0.1 and 1 µg/l* $HgCl_2$ - 4 months) the covering epithelium is completely destroyed and atrophy of muscle bundles was observed in all specimens (Fig. 9). In the connective tissue there are lacunae with hemocytes (Fig. 10). An extensive accumulation of mercury was observed on the basal surface of the epithelium and in the external half of the foot at mussels intoxicated with 1 µg/1 $HgCl_2$. Basally the deposits formed a dark band below the epithelium (Fig. 11). Also, characteristic to this experimental variant is the mercury accumulation at the basis of hemolymph vessels (Fig. 12).

Histopathological changes induced by a high concentration of metal (*5 µg/ml* $HgCl_2$) within short time (*4 days*) are similar to those produced by long time intoxication with lower doses.

Fig. 10. – Lacunae with hemocytes in the connective tissue of the foot. 1 µg/l $HgCl_2$ - 4 months. Scale bar = 50 µm.

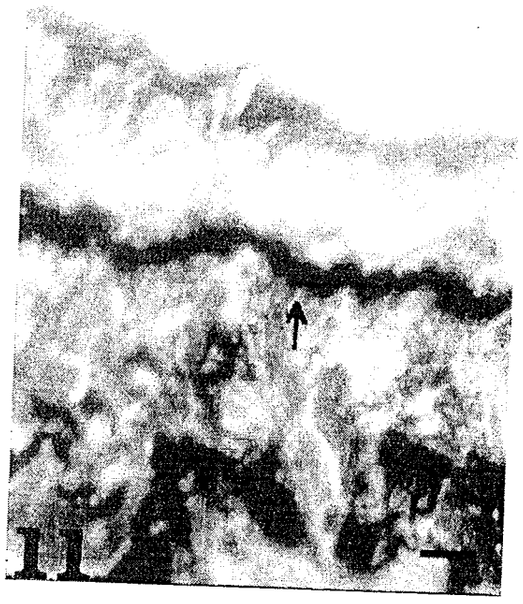

Fig. 11. – An extensive accumulation of mercury on the basal surface of the epithelium (arrow) and in the external half of the foot at mussels intoxicated with 1 µg/l $HgCl_2$ - 4 months. Scale bar = 50 µm.

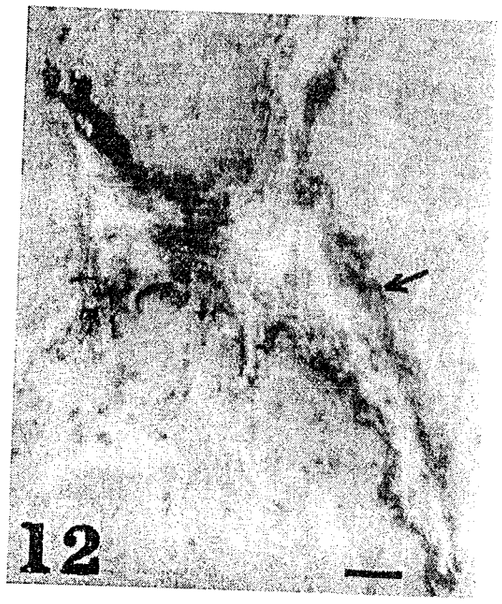

Fig. 12. – Mercury deposits at the basis of hemolymph vessel (arrow). Mussel intoxicated with 1 µg/l $HgCl_2$ - 4 months. Scale bar = 50 µm.

DISCUSSION

A feature of the present study is the correlation between the histopathological changes induced by mercury and localization of this metal in the foot of *Anodonta piscinalis*.

Disruption of the ventral epithelium of the foot may provide useful information for environmental monitoring as lesions probably occurred in direct response to contaminant exposure in the absence of metabolic modification. A similar effect was observed in the foot of *Macoma carlottensis* exposed to natural and anthropogenic stress (3) and in the siphons of *Macoma balthica* exposed to copper (8). On the contrary, the histological structure of the foot in the gastropod *Viviparus viviparus* is unaffected by the lead pollution but the phospholipid and sulpholipids concentration decrease (2).

Atrophy of muscle bundles and pyknotic nuclei has also been observed in *Mytilus edulis* exposed to heavy metals (15).

Previous studies have shown that the lowest accumulation of heavy metals was in the adductor muscles and foot (4, 9, 11, 13, 16). This is consistent with our results which showed few mercury deposits in the muscle tissue of the foot.

Our microscopic findings demonstrated that mercury was accumulated on the basal surface of the epithelium and in the external half of the foot. This is consistent with the result on the crustacean species, *Crangon crangon,* which was shown to accumulate mercury below the tubular epithelium of the hepatopancreas (1).

The hemocytes with mercury deposits were also observed in *Crangon crangon* inside the hemolymph channel (1) and in *Anodonta piscinalis* in the connective tissue of female gonad (17) and in the hemolymph extracted from adductor muscle (18). Among the many functions attributed to bivalve hemocytes is that of a lysosomal function. Hemocytes actively scavenge the blood sinuses, phagocytizing particulate and dissolved foreign materials including pollutants (14). The most probable transport system is composed of two pathways: 1- the hemocytes somehow transfer their wastes to the kidney or other epithelial cells for conveyance to the outside or 2 - the hemocytes traverse the epithelium to the outside and either void their contents and recross the epithelium to hemolymph or are lost to the animal completely (14). Presence of a great number of hemocytes in the outermost zone of the foot possibly indicates elimination of these cells. Bright and Ellis (3) also observed large deposits of fine basophilic granules between peripheral musculature of *M. carlottensis* foot. These appeared to be extracellular. George and Pirie (9) demonstrated that zinc in the mantle and muscle (possibly in hemocytes) remain for a long period. Our results support these findings, showing that there are mercury deposits in hemocytes and in the connective tissue of the foot after four months exposure.

In conclusion, numerous aspects of histopathological changes were noticed, especially, disruption of the surface epithelium and connective tissue architecture between muscle bundles, abnormal concentration of hemocytes in the outermost zone of the foot and atrophy of muscle bundles. Autometallographically silver-enhanced mercury deposits were localized by light microscopy in: apical pole of epithelial cells, on the basal surface of the epithelium, in the connective tissue around muscle bundles, between muscle fibers, at the basis of hemolymph vessels and in the hemocytes.

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GENETIC CONDITIONING IN BECKER-KIENER PROGRESSIVE MUSCULAR DYSTROPHY

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Becker-Kiener progressive muscular dystrophy (BMD) is an X-linked recessive (gonosomal recessive) disease. The gene is located on the Xp21 chromosome. BMD strictly affects the male sex; the onset age is after 5 years, muscular fatigue occurring after 11-13 years. The disease has a slow evolution, patients becoming wheelchair bound between 20 and 30 years. Treatment is only prophylactic. Detecting of carriers and competent genetic counselling have proved to be efficient. Genetic therapy is expected to discover an effective treatment of this disease.

Becker-Kiener progressive muscular dystrophy (BMD) is an X-linked recessive (gonosomal recessive) disease (1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

The features of the disease are:

- expression strictly limited to the male sex;
- progressive muscular atrophy;
- slow evolution compared to that of Duchenne progressive muscular dystrophy;
- increased CK levels;
- intrafamilial variability of the disease evolution and severity.

The onset age is after 5 years, and muscular fatigue occurs after 11-13 years. BMD patients become wheelchair bound between 20 and 30 years (2, 3, 6, 9, 10).

We proposed the genetic study of this form of muscular dystrophy because since the disease is usually diagnosed late in life, it requires a correct prophylactic attitude in order to detect potential carriers and to offer competent genetic counselling.

MATERIAL AND METHODS

In this study we investigated in the Centre of Neuromuscular Pathology "Horia Radu" Vâlcele, Covasna Department, a number of 17 families in which the BMD gene is phenotypically expressed.

The methods used were:

- the method of familial inquiry, using the genetic record and genealogical tree construction in order to establish the inheritance mechanism of the disease.

The patients were investigated paraclinically through serum enzymogram, electromyogram (EMG) and muscular biopsy.

Serum enzymogram consists in determining the enzymatic activity of serum creatine phosphokinase (CK) by Foster G method (1970); aldolase (ALD) by Bruns F method (1954) and glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) by Reitman F method (1957).

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The normal serum activity values at 25°C are the following:
CK = 50 IU/l; ALD = 0.5 - 3.1 IU/l; GOT and GPT = 12 IU.

In order to confirm the BMD myogenic background we performed an EMG and muscular biopsy examination.

RESULTS AND DISCUSSION

Serum enzymogram of BMD patients exhibits a five-fold serum CK increase over the normal limit. This increase in CK enzymatic activity parallels the evolution and the mobility degree of the disease. A moderate increase in serum aldolase, GOT and GPT levels is also found, compared to the normal values.

EMG in BMD patients shows the existence of short duration, small amplitude action potentials. Action potentials are polyphasic and interferential (Fig. 1). The conduction speed of sensitive and motor nerves is normal. The EMG changes confirm the myogenic background of the disease.

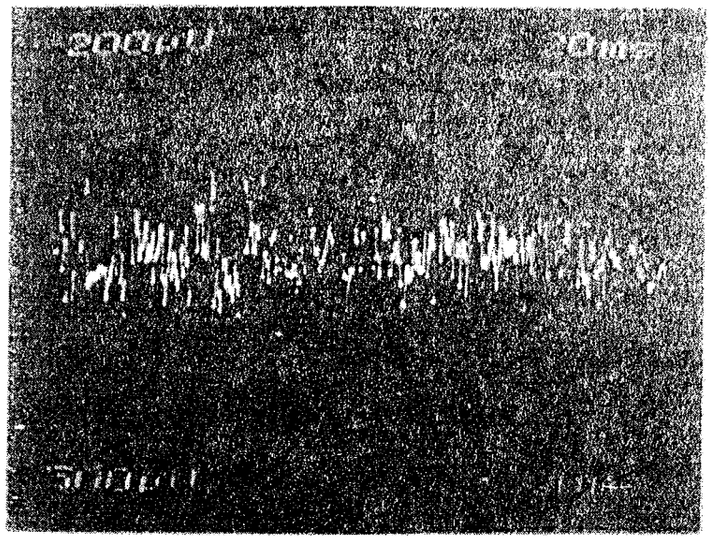


Fig. 1

In the case of BMD patients' muscular biopsy, histopathological findings show an abnormal size variation of disseminated or assembled atrophic and hypertrophic muscular fibres, necrosis and rare regenerative fibre areas. Depending on the stage of the disease, lipidic metaplasia and endomysial and perimysial conjunctive tissue proliferation is found (Figs. 2, 3, 4).

The BMD gene is recessive and it is located on the short arm of the X chromosome: Xp2l, at the same locus as the DMD (Duchenne progressive muscular dystrophy) gene, the two genes being allelic and controlling the synthesis of a cytoskeletal protein in the striated muscle - dystrophin (1, 9).

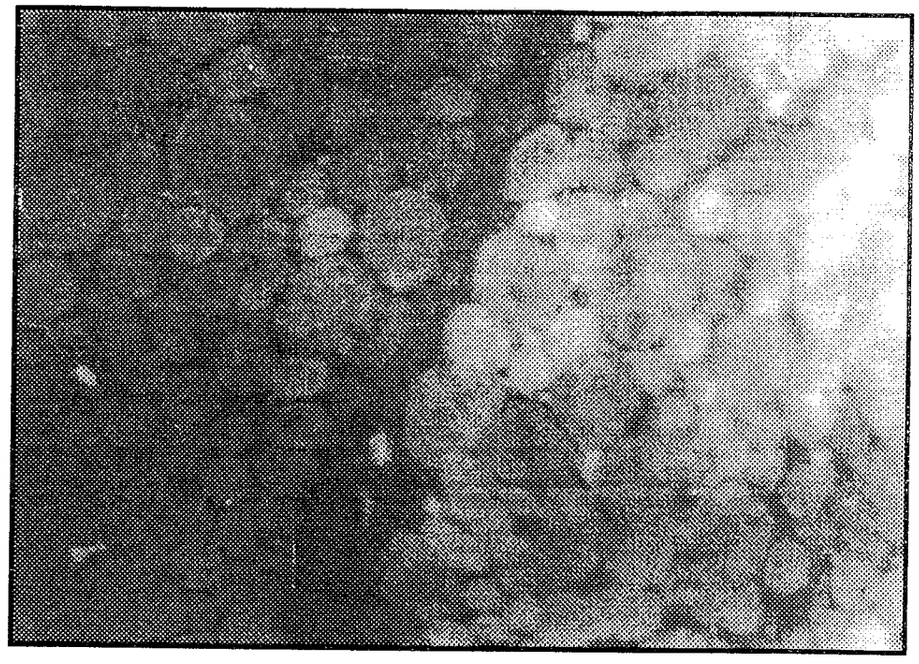


Fig. 2

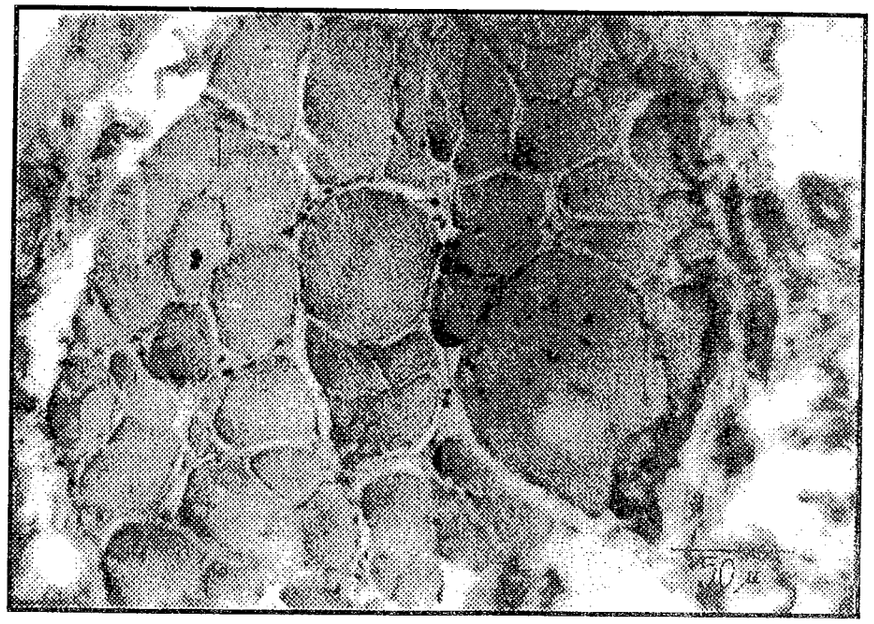


Fig. 3

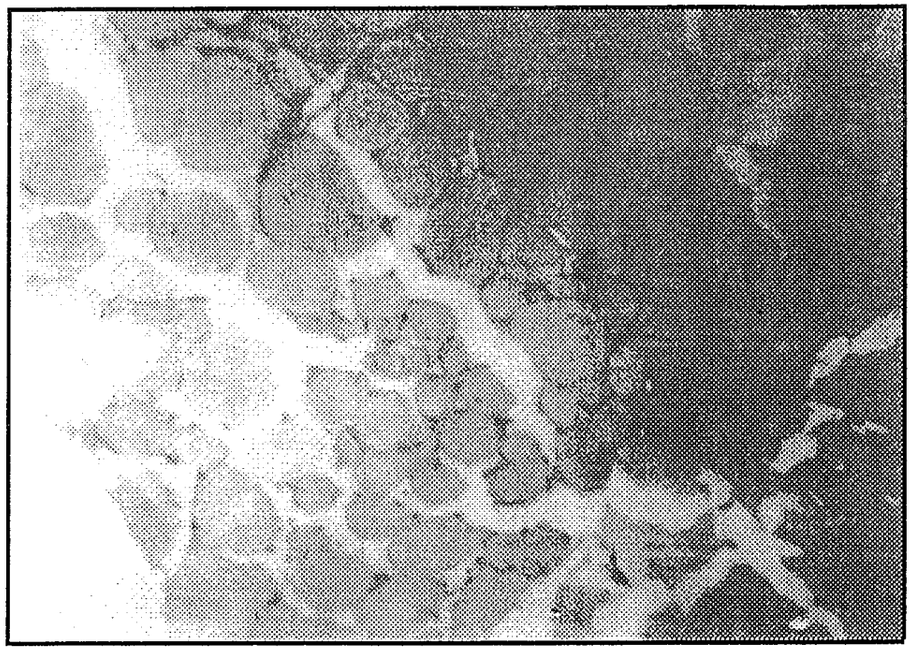


Fig. 4

Dystrophin has portions homologous to some cell plasmalemma proteins, which play a role in Ca^{2+} transport through the sarcolemma (1, 6, 9).

The mutations in the dystrophin gene are mostly intragenic deletions (less frequently duplications or inversions), leading to severe clinical disorders - DMD, or less severe - BMD (l, 2, 4, 9).

If there is a complete deletion in the dystrophin gene, its synthesis will no longer occur, and the phenotypic expression will be that of DMD (l, 2, 4, 9).

If the mutation consists in the deletion of a small number of exons or a small amplitude duplication, it will result in the synthesis of dystrophin with a shorter or a longer chain, that will be incorporated into the sarcolemma, BMD being phenotypically expressed (1, 2, 4, 6, 9).

In 80% of the BMD cases, the presence of a normal dystrophin molecule can be detected, but it is synthesised in a low amount (l, 2, 4, 9).

We present now the genealogical trees of the most interesting cases studied.

In the family DM, the gene is phenotypically expressed through four generations in four men: the proband, a mother's brother, two brothers of the maternal grandmother and a second maternal cousin (Fig. 5).

The onset age of the disease in the proband is 14 years, the symptoms being muscular weakness of the pelvic girdle, tiring ambulation and orthostatism, calves hypertrophy. The serum enzymogram of the proband: CK = 289.5 IU; ALD = 7.5 IU/l; GOT = 19 IU; GPT = 16 IU; and EMG indicate a myogenic interferential

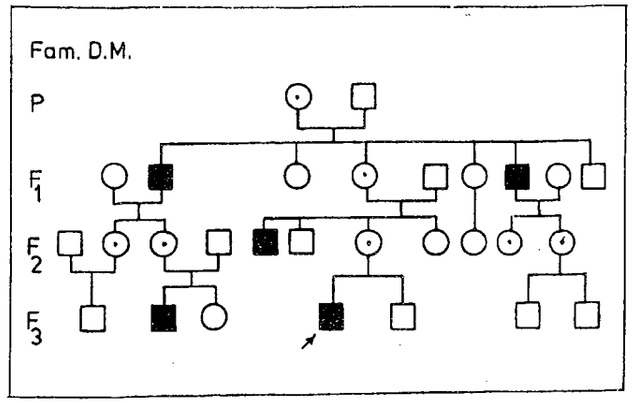


Fig. 5

pathway. These results as well as the slow evolution confirm the BMD clinical diagnosis. From the analysis of the genealogical tree of this family, gonosomal recessive inheritance and complete penetrance of the BMD gene are deduced.

By studying the three generational pedigree of family CI, the Becker-Kiener phenotype is found in four males. The study of this pedigree confirms the recessive X-linked inheritance of the BMD gene in this family. The mother of the four men affected is a definite carrier, as well as the daughters of the two married men affected. In this family the onset age of the disease ranges between 15 and 20 years, with a large variability of its evolution (Fig. 6).

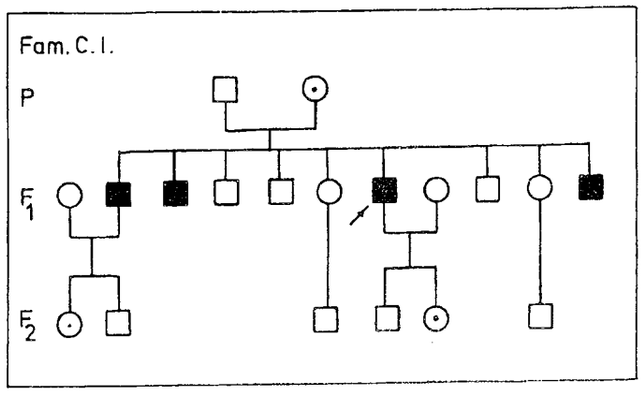


Fig. 6

In the proband, the first symptom of the disease is muscular weakness, and moderate calves hypertrophy occurs at the age of 18 years. Paraclinical investigations show: CK = 253.2 IU/l; ALD = 8.5 IU/1; GOT = 17 IU; GPT = 14 IU; EMG exhibits a myogenic interferential pathway and confirms the BMD diagnosis.

In family IA, the mother and the grandmother of the proband are definite carriers, the proband and three brothers of the proband's mother being affected. In this family the BMD gene has a recessive X-linked inheritance as well, affecting exclusively the males (Fig. 7).

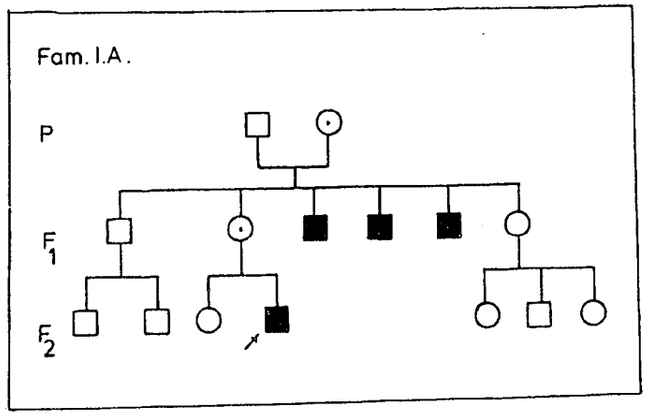


Fig. 7

The clinical picture, the late onset of the disease (11 – 17 years), the paraclinical investigations of the proband: CK = 382.3 IU/l; ALD = 7.3 IU, as well as EMG with myogenic interferential pathway and the histopathological findings of muscular biopsy confirm the BMD diagnosis.

Of the 13 members of three generational Family FA, three of the six sons are affected by BMD: the proband, a brother and a nephew (a sister's son) of the proband. The mother and the sister of the proband are carriers, having two sons, two brothers and a son, respectively, affected (Fig. 8).

The BMD diagnosis of this family patients is confirmed by the clinical picture, the onset age (23 years), the serum enzymogram: CK = 205 IU/l; ALD = 6.27 U/l; GOT = 164; GPT = 14 U; EMG indicating a myogenic interferential pathway in the proband.

The study of the genealogical trees of the 17 families investigated shows clearly the gonosomal recessive inheritance of the Bercker-Kiener progressive rnuscular dystrophy gene, which is demonstrated by the fact that only the boys are affected.

The risk of BMD recurrence is 50% (1:2) of the male newborns of a carrier mother. In the rare case when the affected father marries a descendant, in the first generation his daughters will be carriers, the boys being unaffected since they will receive the X chromosome from their mother.

In the second generation, the daughters of this man have a 50% chance (1: 2) to transmit the Becker-Kiener gene to their sons. The BMD populational incidence is 1: 34, 000 male newborns.

As it is an invalidant disease in spite of its late onset and slow evolution, BMD has serious economic and social implications.

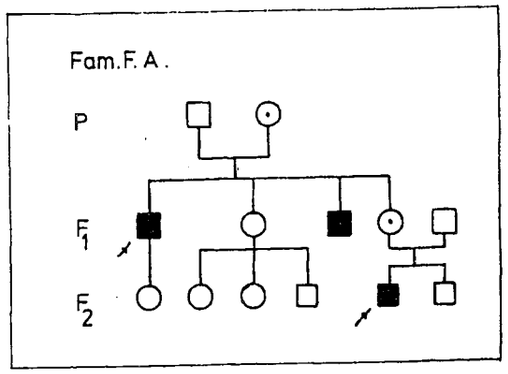
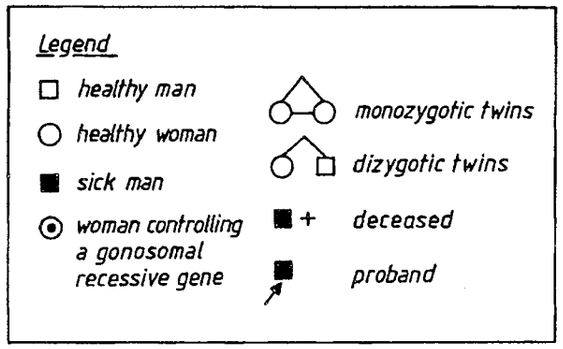
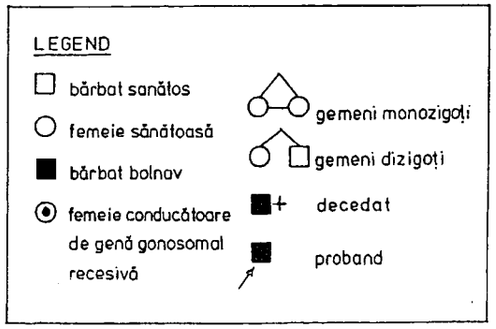


Fig. 8





BMD treatment is unknown. All forms of treatment tried to date: myoblast transplantation, gene transfer by means of adenoviruses, ATP and phosphocreatine administration, hypervitaminisation, have not proved to be efficient.

In our country orthopedic physiotherapy is applied, which tries to avoid as much as possible muscular fatigue and tendinous retractions and spine deformity. Effective prophylaxis consists in detecting carrier girls and offering competent premarital genetic counselling.

CONCLUSIONS

1. The BMD mutant gene is located on the X chromosome: Xp21, at the same locus as the DMD gene, affecting exclusively the male sex and having a slow evolution.

2. Paraclinical and genetic investigations performed in 17 families confirm the clinical BMD diagnosis.

3. In BMD, there is a five-fold increase of CK serum levels over the normal limit, which parallels the evolution of the disease. Aldolase, GOT and GPT activities are moderately increased.

4. EMG changes demonstrate the myogenic background of the disease. The histopathological findings confirm, depending on the evolutive stage of the disease, specific BMD muscular changes.

5. The genealogical tree construction of the families investigated proves the X-linked recessive inheritance of BMD.

6. BMD treatment is palliative, but competent genetic counselling can offer adequate prophylaxis.

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SOME INTERACTIONS OF MANITOL, SORBITOL AND THEIR HEXAACETYLATED DERIVATIVES WITH THE METABOLISM OF MUSCLE CELL

ŞT. AGRIGOROAEI, P. ROTINBERG, SMARANDA KELEMEN, I. NEACŞU, V. CRĂCIUN, MARGARETA CRĂCIUN

The effects of manitol (M), sorbitol (S), hexaacetylmanitol (HAM) and hexaacetylsorbitol (HAS) upon the glucidic, lipidic and proteic tissular metabolism of frog striated muscle fibers - incubated for 3 hours, at room temperature, in a physiological solution containing 5 mg/100 ml of each agent tested - have been investigated. Manitol was seen as inducing a slight increase of glycogen's anaerobic splitting along with increase of the muscle lactic acid; also, a significant stimulation of the synthesis of free fat acids and of proteins synthesis was oberved. Sorbitol inhibited strongly glycogen's anaerobic splitting, reduced the muscular lactic acid, and increased quite considerably the synthesis of both free fat acids and proteins. HAM and HAS diminished glycogen's anaerobic scission, in parallel with intensifying significantly metabolization of the lactic acid. No important effects have been observed upon either proteins or total muscular lipids, stimulating to a lower extent - comparatively with M and S - the synthesis of the free fat acids from the tissue.

Hexahydroxylic alcohols (hexites) - having a chemical structure similar to hexoses - are natural products to be met in a free state (but also as esters or other combinations) in fruit or juices of both terrestrial plants and marine algae (11), (13). The properties they possess permit them multiple interactions with various living systems, which contribute to their large utilisation in quite various domains, such as microbiology, pharmacology, etc. (5), (7).

The utilization of manitol (M) as an osmotic non-electrolytic diuretic (for correcting oligoanuritis provoked by various causes) is well-known (3), (5), as well as in treating cerebral oedema, etc., or in diagnosis (differentiation of functional renal insufficiency from the organic one) (10). Manitol has been also employed in the preparation of certain derivatives with cytostatic action (such as manomustine, mytobromite, cytostope, etc.) as carrier of some active groups, which facilitates transport and attenuates their toxicity (4), (6), (14).

In its turn, sorbitol (S) was found as an easily metabolizable energetic substrate, independent of insuline, being utilized as "sugar" for the diabetics ("sionone") and also in parentheral alimentation (in surgical interventions). Its antiketogenic and hepatoprotecting action has been also evidenced (7), (8), (10).

In spite of all these observations, the interactions of such polyols with the cellular metabolism have not been studied thoroughly, which explains the general idea that manitol (unlike sorbitol) would not be metabolized in the animal organism, and also the scarce data existing on the metabolic interactions of sorbitol itself.

Even less studied have been the metabolic interactions of these products' hexaacetylated derivatives (i.e., hexaacetylmanitol - HAM and hexaacetylsorbitol - HAS), although they are expected to show - due to their chemical properties (a significant amount of acetyl groups, first) - physiological actions different from those of manitol and, respectively, sorbitol.

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Consequently, it is our belief that more profound investigations devoted to such problems might suggest, too, their new possible utilizations in various domains.

MATERIAL AND METHOD

All experiments have been performed *in vitro* on frog striated muscle tissue, incubated at room temperature (+ 22°C) for three hours, in physiological solutions containing 5 mg/100 ml from each of the four products considered for the study.

In the case of physiological solutions containing HAM and respectively HAS, they have been first dissolved in a small amount of propylenglycol (PGL) (0.5 ml for 100 ml physiological solution).

After the incubation period, glucose, lactic acid, total lipids and the free fat acids, contractile, non-contractile and total proteins have been taken over - by specific methods - from the tissue samples, their concentrations being determined photocolorimetrically.

The proteins have been determined by the Lowry (9) method, total lipids - by the Woodman-Price (15) one, the free fat acids by the Baretto-Mano (2) method, glucose by the method employing o-toluidine (12), and the lactic acid by the Barker-Summerson method (1).

The results have been reported at 100 g fresh tissue, being expressed as percent variations versus the values registered with the reference sample.

In the case of HAM and HAS, the specific effect of PGL - as a matter of fast, very low - has been also considered.

RESULTS

The experiments performed as described provided a series of preliminary information on some important and various interactions of the products under test with the cellular intermediary metabolisms (Table 1).

Table 1

Effects of the agents tested upon some parameters of the intermediary cellular metabolism (Percent variations *versus* the reference sample, for 100 g fresh tissue)

Agent	Glucose	Lactic acid	Total lipids	Free fat acids	Total proteins	Contract. proteins	Noncontract. proteins
Manitol (M)	−3.28	+9.68	−3.40	+27.50	+11.80	+9.20	+14.80
Sorbitol (S)	−20.78	−7.82	−4.60	+31.70	+15.50	+13.80	+17.40
Hexaacetyl-manitol (HAM)	−8.48	−45.85	+0.81	+9.50	+0.50	+4.10	−2.90
Hexaacetyl-sorbitol (HAS)	−39.36	−55.40	+0.90	+8.30	+0.90	+7.40	−6.40

For the samples not treated with the agents taken into study (the reference samples), the following values of the metabolic parameters considered have been found for 100 g fresh tissue: 48.81 mg for glucose, 74.08 mg for the lactic acid, 2.38 g for total lipids, 0.422 g for the free fat acids, 7.36 g for total proteins, 3.91 g for contractile and 3.45 g, respectively, for noncontractile proteins.

In the samples treated with M, the following variations have been observed, comparatively with the normal values: increase of muscular glucose (with 3.28%) and a more pronounced increase of the lactic acid (with 9.68%), a slight decrease of total lipids (with 3.40%) and a very significant increase of the free fat acids (with 27.50%), an increase of total proteins (with 11.80%), more significant being the increase of noncontractile proteins (with 14.80%), comparatively with that of contractile proteins (with 9.20%).

In the case of S - treated samples, nevertheless, a pronounced decrease of muscular glucose has been recorded (with 20.78%), accompanied by a reduction of the lactic acid as well (with 7.82%). The effects of S upon muscular total lipides (a 4.60% reduction) and also upon the free fat acids (a 31.70% increase) are of the same type with those of M, although more pronounced.

Also, S induces a significant increase of muscular total proteins (with 15.50%), more important being again the increase of soluble proteins (with 17.40%), comparatively with that of contractile proteins (with 13.80%).

As to the hexaacetylated derivatives of such polyols, the observation was made that HAM induces quite an important reduction of muscular glucose (with 8.48%), accompanied by a very pronounced decrease of the muscular lactic acid (with 45.85%). Nevertheless, this product does not modify considerably the amount of muscular total lipids, leading to a lower increase of the free fat acids (with 9.50% comparatively with that determined by M. Nor are the total muscular proteins significantly modified, the slight increase of noncontractile proteins (with 2.90%) being compensated by a slight decrease of the contractile ones (with 4.90%).

In its turn, HAS has a very pronounced effect of reducing the amount of muscular glucose (with 39.36%), as well as of the amount of lactic acid (with 55.40%). Nor does HAS modify significantly the amount of total muscular lipids, leading, nevertheless, to an increase of the free fat acids (with 8.30%). In this case, too, muscular total proteins are not considerably modified, a slight reduction of noncontractile protein (with 6.40%) being compensated by an increase of contractile protein (with 7.40%).

DISCUSSIONS AND CONCLUSIONS

The results obtained by us invalidate first the ideas presented in the literature of the field (5), (10) - according to which manitol would not be metabolized in the animal organism.

The increase of muscular glucose induced by this product can be explained exclusively by stimulation of glycogenolyse (glycogen's anaerobic splitting). In its turn, such glycogenolyse stimulation might by explained by the reduction of cellular oxidative processes (the competition between glycogenolyse and oxidation),

by the utilization of cellular oxygen to the very transformation of manitol into D-mannose and then in glucose-6-phosphate. Therefrom would result, concomitantly, an increase of cellular glucose as well as an increase of the lactic acid, the oxidative degradation of which to CO_2 and H_2O would be diminished. In the case of the muscular tissue, no inhibition in the synthesis of glycogen from lactic acid may be assumed, as the muscular tissue is not capable of such a synthesis.

On the other side, the slight decrease of muscular total lipids by M would explain only partially the important increase of the muscular free fat acids, through the lipolysis of triglycerides. It might be therefore accepted the idea that M has also a considerable effect in stimulating the synthesis of free fat acids as such.

The observation was also made on manitol's involvement in the biosynthesis of cellular proteins (anabolizing effect), the recorded increase of muscular total proteins being mainly due to the intensification of the soluble proteins' synthesis.

In the case of S, known as an easy-to-metabolize energetic substrate, the significant reduction of muscular glucose assumes inhibition of glycogen's anaerobic splitting, as determined by the very presence of sorbitol in the tissue, an inhibition that might induce increases of the oxidative processes and, consequently, reduction of the amount of muscular lactic acid.

Sorbitol's lipolytic effect is slightly higher than that of manitol, to which an increased effect upon the synthesis of the free fat acids is associated as well. In the case of S, too, stimulation of proteins' biosynthesis stronger than manitol's was recorded, attained, nevertheless, first by the stimulation of noncontractile proteins' synthesis.

It is worth mentioning the fact that sorbitol's metabolic effects differ from those of M mainly as to their interactions with the glucidic metabolism, one knows that the interactions with the lipidic and, respectively, the proteic metabolism differ only quantitatively.

As to the hexaacetylated products of M and S, one should observe the occurrence of their interactions with the glucidic metabolism of the type of the sorbitol's ones quantitatively, even more pronounced, along with their much weaker effects upon both proteic and lipidic metabolism.

Therefore, manitol's and sorbitol's acetylated products reduce significantly glycogen's anaerobic splitting, intensify considerably glucides' oxidative degradation and stimulate to a lesser extent the synthesis of the free fat acids in the tissue, without modifying extensively muscular total lipids and proteins.

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IN VITRO BIOCOMPATIBILITY EVALUATION OF COLLAGEN-BASED SPONGES

LUCIA MOLDOVAN, ANCA OANCEA, OTILIA ZĂRNESCU AND G. BARBOI

Epithelial cells were cultured in three-dimensional reconstituted matrices made of collagen or collagen and glycosaminoglycans. The reconstituted matrices were conditioned as sponges by lyophilization of the type I collagen solution and of the type I collagen-chondroitin 4 sulfate-keratan sulfate mixture. Epithelial cells placed on both types of sponges attached to them and migrated into these reconstituted matrices. We also observed that the collagen-glycosaminoglycan sponges allowed a greater cellular invasion than the collagen sponges.

In the last decade, the need to replace organs and tissues has stimulated an active research for obtaining some synthetic and biologically derived materials for human implanting. In order to be successful, the materials must be nontoxic, well tolerated by the body, compatible with blood, nonclotting and relatively stable in a biological environment (1), (2). These are the reasons why the application of collagen alone or associated with glycosaminoglycans for obtaining biomaterials used as subcutaneous implants, haemostatic agents and as replacement of dermis in skin wounds and burns was extensively investigated (3), (4).

Due to this widespread clinical use, the biocompatibility of collagenous materials was evaluated by "in vitro" cellular responses (5) as well as "in vivo" tissue reactions (6).

In previous papers we investigated the behaviour of some cell types grown on membranes (7) and sponges (8) prepared from bovine dermal collagen. The results demonstrated that the experimented materials were biocompatible and had a biostimulating effect on the growth of cells in culture.

The aim of the present paper was to investigate the "in vitro" biocompatibility of collagen sponges and collagen-glycosaminoglycan sponges (composed of type I collagen: chondroitin 4 sulfate: keratan sulfate in a weight ratio of 4: 0.66: 0.33) in epithelial cell culture.

MATERIALS AND METHODS

PREPARATION OF COLLAGEN-BASED SPONGES

Type I collagen used in the present study was prepared by us from bovine corneas by pepsin treatment as described previously (8).

Chondroitin 4sulfate and keratan sulfate were extracted from bovine tracheal cartilage by papain treatment and ethanol precipitation and purified by ion-exchange chromatography (9),(10).

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Sponges were prepared by freeze-drying of the collagen solution (0.8%, w/v) and of the collagen-glycosaminoglycan mixture (4:1, w/w), at pH 7.0. The glycosaminoglycan fractions contained chondroitin 4sulfate and keratan sulfate (2:1, w/w).

STERILIZATION METHOD

The sponges were sterilized by gamma ray (60Co) treatment (doses between 7 and 100 KGy).

The sterilization of the products was evaluated by determination of the following microbiological characteristics: total bacteria count, moulds and yeasts content, *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* presence (11).

CELL CULTURE

In order to investigate the biocompatibility of collagen-based sponges we have used a calf kidney epithelial cell line. The sponges were left for 24h at 37 °C, overlaid with MEM medium containing 10% fetal bovine serum. The final period of cell cultivation in the presence of collagen-based substrates was of 96h at 37 °C.

HISTOLOGICAL EXAMINATION

For light microscopy, sponges were fixed in Bouin fluid with 20% DMSO, washed, dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin wax. The sections were stained by Von Gieson method for collagen.

RESULTS AND DISCUSSION

Type I collagen is an adequate molecule for manufacturing biomaterials, owing to its biological properties. In the present study we have used this type of collagen extracted from adult bovine cornea by a non-denaturing method and lyophilized as sponges.

In order to obtain collagen-glycosaminoglycan sponges, type I collagen solution was combined with chondroitin 4sulfate and keratan sulfate in a weight ratio of 4:0.66: 0.33 and lyophilized.

Collagen-based sponges were sterilized by exposure to a gamma-ray dose between 7 and 100 KGy. And then their microbiological characteristics were determined. In order to verify whether the applied gamma-ray doses could modify the structure of collagen, we have evaluated the free hydroxyproline in the sterilized samples (Table 1). The results obtained show that the 8-15 KGy dose is effective for sterilization and causes no damage to the protein structure. A significant increase in free hydroxyproline was observed only in the sample exposed at a 100 KGy dose.

So, we consider that a 15 KGy dose is sufficiently powerful for sterilization of collagen sponges and is certainly devoid of degradative effects.

Macroscopically, the two types of sponges were similar, with a microporous structure. The macroscopic appearance is a direct consequence of the intrinsic fibrillary structure of the non-denatured protein (12). It has been demonstrated that chemical and physical structures of collagen such as forming fibrils, native or denaturated, cross-linked or not, have marked influences on the morphology and physiology of cells (13), (14).

Table 1

Effects of various gamma-ray doses on microbiological characteristics and composition of collagen-based sponges

Dose (KGy)	Total bacteria (colonies/gram)	Moulds and yeasts content (colonies/gram)	Free hydroxyproline (μg/100g)
7.0	27	5	–
12.5	5	–	–
25.0	–	–	7.3
50.0	–	–	34.1
100.0	–	–	110.5
Control*	76	24	–

*Untreated by gamma-ray

We have investigated the "in vitro" biocompatibility of sterilized collagen-based sponges in epithelial cell culture. Histological examination clearly indicated the penetration and invasion of the cultured cells into the sponges.

After 48h incubation the epithelial cells are seen as cellular groups of various sizes at the surface of the collagen sponge (Fig. 1). At 96h, the cells are spread both at the periphery and inside the sponge (Fig. 2). Epithelial cells inside the collagen sponge were either round or oval, having a nucleus with granulous chromatin.

The epithelial cells cultured for 48h on collagen-glycosaminoglycan sponges are disposed as a monolayer on their fibrillar surface (Fig. 3). After 96h culture, the cells had migrated through the sponge and attached to its alveolar structure (Fig. 4).

Histologically, both types of sponges represent a support for epithelial cells adhesion and growth. We have observed that the cells infiltrated much better into collagen-glycosaminoglycan sponges as compared to the collagen sponges.

The ability of these sponges to be good supports for epithelial cell culture make them convenient in obtaining the artificial tissue substitutes. It has already been demonstrated the biocompatibility between fibroblasts and a new type of biomaterial for artificial skin obtained by combining fibrillar collagen with gelatin (15).

Fig.1. – Light micrograph of epithelial cells cultured on the collagen sponge for 48h; cells are located as groups of various sizes at the surface of the sponge. (×100).

Fig. 2. – Epithelial cells cultured on the collagen sponge for 96h; cells are disposed at the periphery and inside the sponge. (×100).

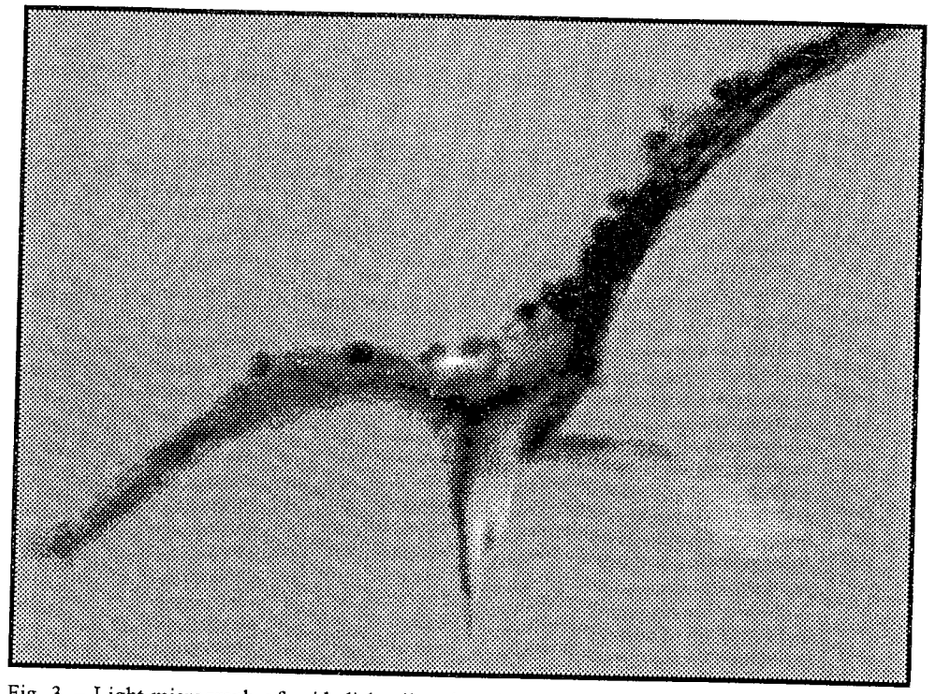

Fig. 3. – Light micrograph of epithelial cells cultured on the collagen-glycosaminoglycan sponge for 48h; cells are disposed as a monolayer at the surface of the sponge. (×200).

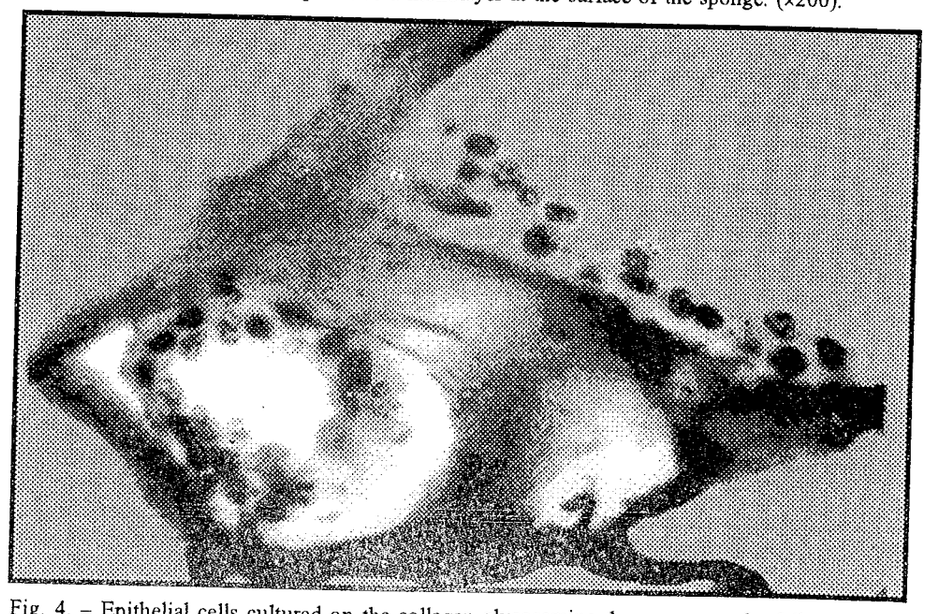

Fig. 4. – Epithelial cells cultured on the collagen-glycosaminoglycan sponge for 96h; cells had migrated through the sponge and attached to its alveolar structure. (×200).

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INFLUENCE DES PULVÉRISATIONS INSECTICIDES SUR LA FAUNE UTILE

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En plein champ, on a expérimenté différents insecticides et mélanges des produits microbiologiques et insecticides chimiques. Les essais dans les cultures de pomme de terre ont démontré une bonne sélectivité pour les entomophages du sol de la phosalone, furathiocarbe (traitement des tubercules), thiofanox (traitement du sol) etc. La luzerne avec une très riche entomophaune a permis de constater que la phosalone est la plus sélective et le trichlorfon est sélectif. Les pulvérisations dans les cultures du chou sont plus sélectives en comparaison avec les poudrages.
Mot-clés: pulvérisation, poudrage, traitement du sol, entomophages du sol, insecticides.
Influence of Insecticide sprayings on useful fauna
Field experiments were developed with different insecticides and mixtures of microbiological products and chemical insecticides. Tests with potato crop have demonstrated a good selectivity for soil entomophages, of phosalone, furathiocarbe (seed dressing), thiofanox (soil treatment). Alfalfa, with a very rich entomophauna, permitted to establish that the most selective product is phosalone and trichlorfon is selective. The sprayings in cabbage crop are more selective, as compared to dustings.
Key-words: sprayings, dustings, soil treatment, soil entomophages, insecticides.

L'étude de la sélectivité des différentes matières actives ainsi que l'étude des méthodes d'application, est une étape importante pour l'élaboration des systèmes de lutte intégrée. Les données existantes à ce sujet touchent en général le comportement des divers entomophages vis-à-vis des insecticides (HASSAN et al. 1988, BAICU 1990).

La sélectivité des pesticides se manifeste en plein champ, en fonction de la dose et la méthode d'application pour toutes les espèces présentes dans la cultures agricoles. En partant de cette constatation les expérimentations ont été effectuées dans les cultures et on a pris en considération la totalité des arthropodes venant en contact avec les insecticides.

MATÉRIEL ET MÉTHODES

Les expériences ont été effectuées à Bucarest et dans les environs, en utilisant un dispositif expérimental randomisé avec 4 répétitions.
Pomme de terre: on a étudié les pulvérations foliaires, les traitements du sol avec des insecticides granulés et le traitement des tubercules.
Pour établir les influences sur la faune du sol on a utilisé les pièges Barber.
Les insecticides ont été appliqués, pour le contrôle de la doryphore (*Leptinotarsa decemlineata* Say) Luzerne. On a choisi la luzerne pour l'abondance des arthropodes, nusibles et utiles, existants dans cette culture agricole. Dans ce cas on a utilisé des insecticides en mélange avec un produit biologique–Thuringin 6000 (*Bacillus thuringiensis*). Les arthropodes ont été collectés avec un biocoenomètre (filet) après l, 3 et 7 jours à partir de la date de la pulvérisation.
Chou: les pulvérisation insecticides ont été appliquées contre la noctuelle du chou (*Mamestra brassicae* L) et autres ravageurs. On a appliqué les insecticides

par pulvérisations et par poudrages. Après l, 3 et 7 jours on a collecté les arthropodes du sol avec des pièges Barber.
Les comptages et les déterminations des genres et des espèces ont été effectués dans le laboratoire.

Pour interpréter les résultats on a utilisé la méthode exposée antérieurement (BAICU 1987) en fonction du pourcentage de survivants par traitement, selon l'echelle (tableau 1).

En fonction du pourcentage des survivants on a défini la sélectivité des différents produits par la méthode d'application.

Tableau 1

Classification de la toxicité et de la sélectivité
Toxicity and selectivity classification

Mortalité %	Toxicité	Survivance %	Sélectivité
< 20	Non toxique	> 80	Très sélectif
20 – 37	Peu toxique	80 – 63	Sélectif
37 – 63	Modérément toxique	63 – 37	Modérément sélectif
63 – 80	Toxique	37 – 20	Peu sélectif
80 – 100	Très toxique	< 20	Non sélectif

On a utilisé des pesticides formulés.

RÉSULTATS ET DISSCUSIONS

Les données obtenues pendant 3 années dans les cultures de pomme de terre (tableau 2) ont démontré une bonne efficacité de la majorité des insecticides.

Les applications des insecticides granulés au sol, et le traitement des tubercules ne sont pas toxiques pour la faune utile du sol.

Après une seule pulvérisation la deltaméthrine s'est avérée moyennement toxique. L'utilisation répétée de cet insecticide a une action plus forte sur la faune.

La faune comprend Aranea, Formicidae, Collembola, Staphylinidae, Orthoptera, (*Gryllus* sp.) et Carabidae (*Harpalus distinguendus*, *Pterostichus cupreus*, *Anisodactylus signatus*, *Dromius* sp., *Amara* sp.).

Dans la luzerne (tableau 3) une pulvérisation a montré que la deltaméthrine et le diméthoate sont moyennement sélectifs et la phosalone est très sélective.

L'action immédiate du malathion et du disulphotone mocrocapsulés est assez réduite (sélective et très sélective) mais la pression continue de l'insecticide sur la faune peut être plus puissante.

Les insectes les plus affectés par les insecticides expérimentés sont les Coleoptera, Heteroptera et Hymenoptera. Les Araneae et les Diptera ont été moins affectés par les insecticides expérimentés.

Pour le chou, le poudrage avec le mélange méthylchlore + carbaryl est moyennement sélectif ainsi que la pulvérisation avec le mélange carbaryl + lindane.

Tableau 2

Efficacité et sélectivité des différents insecticides – pomme de terre
Effectiveness and selectivity of different insecticides - potato

Insecticide	Dose kg m.a./ha	Doryphore Efficacité %	Faune utile du sol Survivance %	Faune utile du sol Sélectivité
Pulvérisation (1 pulvérisation)				
Trichlorfon (Dipterex 80 PS)	1,2	93,3	80	Sélectif
Deltaméthrine (Decis 2,5 EC)	0,0075	97,3	63	Modérément sélectif
Phosalone (Zolone 35 EC)	0,7	96,2	95	Très sélectif
**Traitement des tubercules*				
Furathiocarbe (Promet 40 SD)	1/t	100	100	Très sélectif
**Traitement du sol*				
Thiofaxox (Decamox 10 G)	0,75	100	100	Très sélectif
Carbofuran (Furadan 10 L)	1,00	100	100	Très sélectif

*Pour la production de semence

Cette constatation est valable pour un seul traitement.

Après plusieurs traitements, l'action est plus nocive.

Les mélanges de *Bacillus thuringiensis* avec 2 g m.a. deltaméthrine ou 15 g fenvalerat et 5 g cyperméthrine sont très sélectifs.

La faune collectée avec les pièges Barber est la suivante: *Araneae*, *Collembola*, *Gryllidae*, *Carabidae* (*Harpalus*, *Amara*); *Staphylinidae*, *Anthicidae*, Formicidae et autres espèces.

Tableau 3

Sélectivité des insecticides pour les arthropodes de la luzerne (1 pulvérisation)
Selectivity of insecticides for arthropodes of alfalfa (1 spraying)

Insecticide	Dose kg m.a./ha	Faune utile foliaire Survivance %	Sélectivité
Trichlorfon (Dipterex 80 PS)	1,2	68,09	Sélectif
Deltaméthrine (Decis 2,5)	0,0075	56,59	Moyennement sélectif
Phosalone (Zolone 35 EC)	0,450	84,23	Très sélectif
Diméthoate (Sinoratox 35 CE)	0,525	57,4	Moyennement sélectif

Les résultats obtenus dans les essais ont montré que la sélectivite des insecticides peut être accrue par la méthode d'application. Les applications de produits granulés ou le traitement des tubercules peut améliorer la sélectivité des produits non sélectifs physiologiquement.

Le poudrage des champs de chou n'est pas recommandable parce que la sélectivité est réduite.

Les matières actives et les doses ont une importance décisive pour la sélectivité. De ce point de vue seulement les mélanges de Thuringin avec des doses très réduites de pyrethrinoides de synthèse sont plus sélectifs que la phosalone (le produit standard).

Tableau 4

Sélectivité des pesticides pour la faune du sol utile de chou (1 pulvérisation)
Pesticides selectivity for useful fauna of cabbage (1 spraying)

Insecticide	Dose kg m.a./ha	Survivance %	Sélectivité
Poudrage			
*Méthylchlore + carbaryl (PEB-4 + carbaryl - 1)	1,3 + 0,1	43	Moyennement sélectif
Pulvérisation			
Lindane + carbaryl (Carlintox 50 PU)	0,1 + 0,4	61	Moyennement sélectif
Bacillus thringiensis (Thuringin) + Deltaméthrine (Decis 2,5 EC)	1 p.c. + + 0,002	90	Très sélectif
Bacillus thuringiensis (Thuringin) + fenvalerat (Sumicidin 20 EC)	1 p.c. + + 0,010	97,5	Très sélectif
Bacillus thuringiensis (Thuringin) + cypermethrine (Arrivo EC)	1 p.c. + + 0,005	99,0	Très sélectif
1,1,1-trichloro-2,2-(p-methylphenyle) etan			

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CONTRIBUTIONS OF ENTOMOPHAGOUS PREDATOR INSECTS IN LIMITING THE OUTBREAK OF *LYMANTRIA DISPAR* L. (LEP., LYMANTRIIDAE)

IRINEL CONSTANTINEANU and RAOUL CONSTANTINEANU

In this paper the authors present the predator complex of the gypsy moth. *Calosoma sycophanta* (larvae), *Dermestes erichsoni* (larvae and adults), *Dermestes lardarius* (larvae) and an unidentified Heteroptera species (adults) are the oophagous predators of the gypsy moth. *Calosoma sycophanta* (larvae and adults), *Calosoma inquisitor* (larvae) and *Silpha carinata* (adults) are larval predators. *Calosoma sycophanta* (larvae) and jackdaw (*Coleus monedula*) are pupal predators. We mention the phenomenon of predatism on gypsy moth females filled with eggs for the first time in science.

The gypsy moth (*Lymantria dispar* L.) is the main defoliator of the oak woods in Romania, with repeated outbreaks, causing heavy defoliations, especially in the southern part of the country. The populations of this defoliator are limited by a parasite and predator complex, which can often contribute to limiting its outbreaks. We established the parasite complex of this defoliator in our previous papers [3, 6].

In this paper we present the entomophagous predators as mortality biotic factors in limiting the populations of this defoliator.

MATERIALS AND METHODS

We carried out our studies in 14 oak woods in the southern part of Romania between 1977-1990. In these woods our investigations were carried out in observation plots. Yearly, during April-August, we watched the activity of the entomophagous predators on gypsy moth populations in all its immature instars and for adult females as well.

In order to establish the activity of oophagous predator we examined groups of 20 oak trees each with gypsy moth egg clusters in each observation plot. We estimated this activity by taking into account the percentage of attacked egg clusters and the average percentage of the eggs destroyed by the oophagous predators in an egg cluster.

According to this percentage, we established four classes of the attacked egg clusters: I 0-25%, II 26-50%, III 51-75% and IV 76-100%. We calculated the attack intensity of these predators by using these classes:

$$I = \frac{(n_1 \times 13) + (n_2 \times 38) + (n_3 \times 63) + (n_4 \times 88)}{N}$$

where :
n_1 = number of egg clusters with an attack intensity of 0-25%
n_2 = number of egg clusters with an attack intensity of 26-50%
n_3 = number of egg clusters with an attack intensity of 51-75%

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n_4 = number of egg clusters with an attack intensity of 76-100%
N = total of analyzed egg clusters

RESULTS AND DISCUSSIONS

In Fig. 1 we present the predator complex of all immature instars and adult females of the gypsy moth.

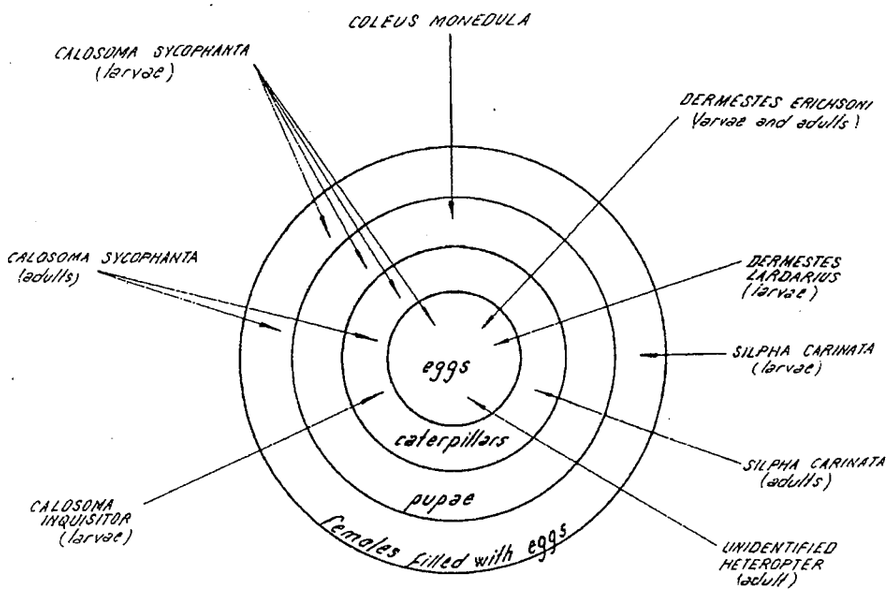

Fig. 1. – The predator complex of *Lymantria dispar* L.

OOPHAGOUS PREDATORS

While the oophagous parasite insects are permanent components of the gypsy moth investigated populations, the oophagous predator insects are not present in these populations every year.

The gypsy moth egg clusters were attacked by *Dermestes erichsoni* Gnglb. and *Dermestes lardarius* L. (ord. Coleoptera, fam. Dermestidae) (larvae and adults), *Calosoma sycophanta* L. (ord. Coleoptera, fam. Carabidae) (larvae). Also, we noticed some unidentified Heteroptera adults, which, probably, sting the gypsy moth eggs, feeding on their contents.

Of the two predator dermestids, *Dermestes erichsoni* was the main oophagous predator met in all investigated woods, while *Dermestes lardarius* was present only in the woods of Dolj and Gorj counties.

Dermestes erichsoni is an univoltin species. In spring the overwintering fecundated females lay 150-200 eggs in the neighbourhood of prey egg clusters of other food sources. The young larvae are hatched at the end of April or the beginning of May. The larval development lasts long time, from May to August, with five or

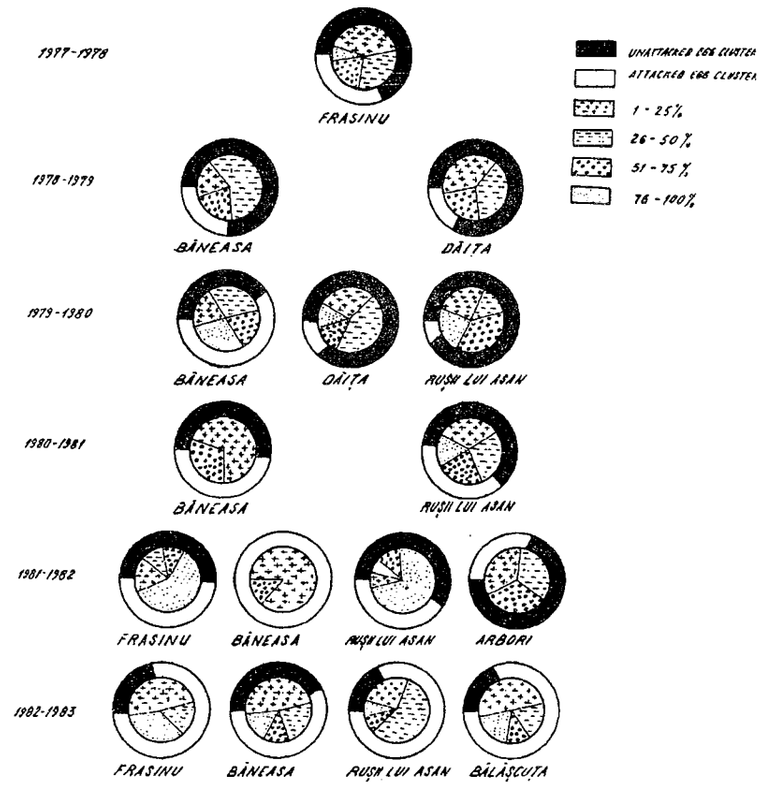

Fig. 2. – Activity of oophagous predators on gypsy moth populations between 1977-1983.

six larval instars. During this period the larvae feed actively on the gypsy moth eggs. Similar observations were made by other authors [7]. At the end of August the larvae transform into pupae. The adults emerge in September.

We noticed that *Dermestes erichsoni* is both an oophagous predator and also a necrophagous species, because it feeds on gypsy moth caterpillars, dead by polyhedrosis, as well as on dead pupae.

From March to May we also identified a spidermite, *Trombidium* sp. (ord. Acari, fam. Trombidiidae) in attacked egg clusters in almost all investigated woods. We tested the role of this spidermite on the gypsy moth egg clusters in our laboratory researches. We put samples of 100 eggs each in 10 Petri boxes, in which a relatively optimum humidity was maintained. We noticed that this spidermite did not feed on gypsy moth eggs. We concluded that this spidermite is an inquilin, which uses the holes made in the egg clusters by the oophagous predators, as its shelters.

We watched the oophagous predator activity in two periods: between 1977-1983 in seven oak woods in Giurgiu county (Fig. 3) and between 1986-1989, with our observations extended to Dolj and Gorj counties.

The first period we analyzed 2695 gypsy moth egg clusters from seven oak woods. Of these 875 egg clusters (35.5%) had been attacked by the oophagous predators. The egg clusters were attacked by the oophagous predators in each of all six prey generations in all investigated woods. The attack intensity varied between 5.71-35.15% (Fig. 2).

In the control plot of Frasinu wood, excluded from chemical applications, during 1977-1983 we noticed that the attack intensity of the oophagous predators reached high values each year. The predator populations developed normally because no chemical applications were made in this wood in the last ten years. There, the defoliator occurred in outbreak (generations in 1977-1978 and 1981-1982). *Dermestes erichsoni* was the main factor in limiting these outbreaks, because it destroyed a quantity of 13.05%.-32.18% eggs. Thus, of the 74 analyzed egg clusters of the 1981-1982 generation, 56% had an attack intensity of 76-100 %.

In Băneasa, Dăița, Bălăşcuța woods, where chemical applications were made, the attack intensity of the oophagous predators was lower because these applications destroyed the predator populations.

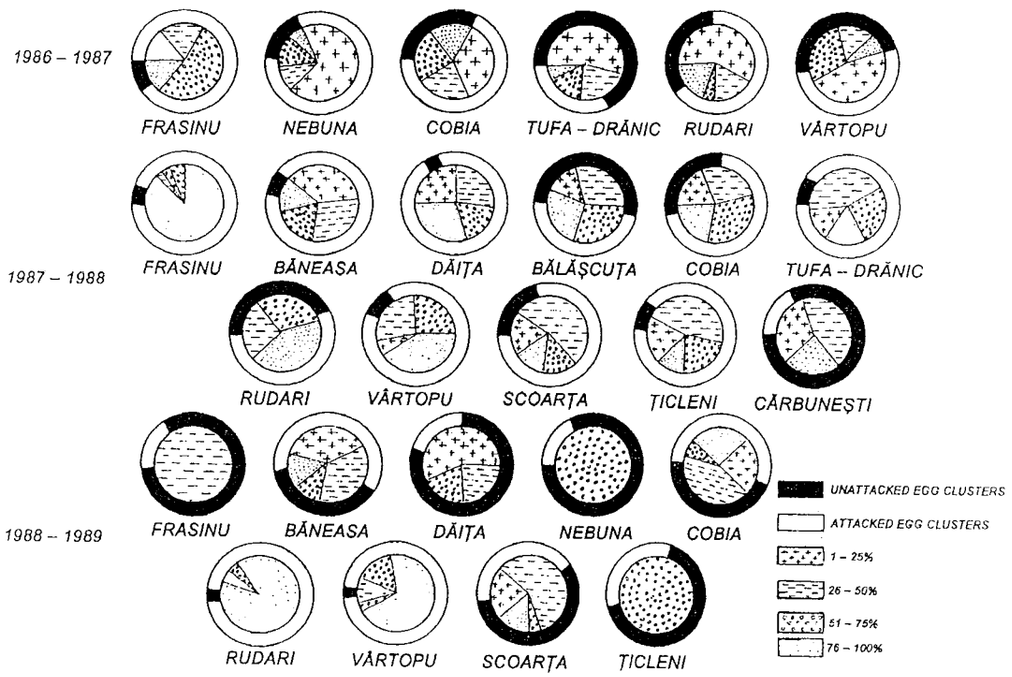

Fig. 3. – Activity of oophagous predators on gypsy moth populations between 1986-1989.

In Rușii lui Asan and Bălăşcuța woods, where microbiological treatments were made, the activity of the oophagous predators remained at an increased level.

During 1986-1989 our researches on the activity of these oophagous predators were extended to other oak woods in Gorj and Dolj counties (Fig. 3). In these oak woods we analyzed 6911 gypsy moth egg clusters of which 3192 were attacked by the oophagous predators (42.2%).

We found the highest attack of egg clusters in 1986-1987 generation of the gypsy moth in Frasinu and Nebuna woods, where the majority of the egg clusters were attacked with an intensity of 51-75%.

In Frasinu wood the attack intensity of the egg clusters in the 1987-1988 generation was of 83%, the majority of these egg clusters having been attacked with an intensity of 76-100%.

In Rudari and Vârtopu woods the attack intensity of egg clusters of the 1988-1989 generation was of 84.6% and of 70.2%, respectively, the majority of them being attacked with an intensity of 76-100%. In these woods microbiological treatments with NPV (nucleopolyhedrosis virus) were made and these maintained the oophagous predator populations at an increased level.

The chemical treatments applied in Țicleni, Nebuna, Dăița woods against the same prey generation led to the diminution of the entomophagous predator population.

We noticed a concentration of dermestids in the observation plots where the virotic epizooties occurred. Similar observations were made by other authors (8). We noticed this phenomenon in Rudari and Vârtopu woods. In these woods we frequently found the dermestid larvae in high concentrations of gypsy moth dead caterpillars at the bases of the tree stems. After depositing the new gypsy moth egg clusters, the dermestid larvae left the virotic caterpillars and began to feed on gypsy moth eggs.The gypsy moth caterpillars destroyed by virotic epizooties represent sufficient food source for the dermestid larvae and provide high concentrations of these predators.

The dermestid larvae took the virus polyhedrons from the dead caterpillars and spread them in the noninfested gypsy moth egg clusters. Thus, the dermestid larvae contribute to the spreading of the virotic epizooties.

PREDATORS OF THE GYPSY MOTH CATERPILLARS AND PUPAE

In the woods investigated we noticed the following predators (Fig. 1):

– caterpillar predators: larvae and adults of *Calosoma sycophanta* L., larvae of *Calosoma inquisitor* L. (ord. Coleoptera, fam. Carabidae) and adults of *Silpha carinata* Hrbst. (ord. Coleoptera, fam. Silphidae);

– pupal predators: larvae of *Calosoma sycophanta* L. and jackdaw (*Coleus monedula* L.) (Cl. Aves, ord. Passeriformes, fam. Corvidae).

Calosoma sycophanta was the most frequent predator of the caterpillar and pupa populations. In Frasinu and Nebuna woods we noticed that the *Calosoma sycophanta* larva destroyed about 15-20 caterpillars daily. This is a voracious species and can kill many gypsy moth caterpillars and pupae although it eats few of them.

In Romania *Calosoma sycophanta* is considered the most frequent predator of the gypsy moth caterpillars and pupae [6]. We also identified it as an oophagous predator (Fig. 4].

The life cycle of *Calosoma sycophanta* is well adapted to that of the gypsy moth [4, 9, 10]. Its adults live 2-3 years. The larval development is of 14 days and the pupal one of 13 days. In only one season a female can lay, by

ovipositor, around 650 eggs in small holes made in the soil. After a couple of days the young larvae climb on the tree stems and feed on gypsy moth pupae and large caterpillars. The third instar larvae pupate in the soil and transform

Fig. 4. – A larva of *Calosoma sycophanta* attacking an egg cluster of *Lymantria dispar* L.

into adults in about two weeks. The adult beetles emerge during the gypsy moth's larval period, in June to feed and reproduce; then they enter the soil in July and stay there until the next spring.

Calosoma sycophanta larva feeds during the day and night. It can eat at least 50 gypsy moth caterpillars of the fifth and sixth instars during two weeks, which represent its feeding period [2].

These predator larvae are more active at the base of the tree stem, where they destroy about 65-70% of the gypsy moth pupae.

The activity of this predator is considerable, attacking many defoliator moth species, with a particular preference for gypsy moth.

The ground beetles, especially those which belong to the genus *Calosoma*, are known as "caterpillar hunters". *Calosoma sycophanta* climbs on the tree stems from the base to the crown to seek its prey. The predator adult catches a caterpillar with its strong mandibles, plunging together with it to the ground. There, it makes

in a part of its contents.

In August, after the gypsy moth females had deposited the egg clusters and died, we did not see adults of *Calosoma sycophanta* in activity.

We made many diggings in the soil, 10-15 cm deep, in the neighbourhood of trees and found many adults of *Calosoma sycophanta*. In 1977 and 1982 the entrance into the aestival diapause occurred because of lack of food and of sultry heats.

In Frasinu wood we also observed the adults of *Silpha carinata*, which

Fig. 5. – A gypsy moth female attacked by a *Calosoma sycophanta* larva.

consumed the gypsy moth caterpillars.

Some bird species play an important role in limiting the gypsy moth populations. Thus, in the first half of July, 1978 we remarked a great number of jackdaws (*Coleus monedula*) in Arbori wood. These consumed many gypsy moth pupae. In the incipient outbreaks, the density of the pupa populations is lower and the insectivorous birds find them with greater difficulty.

Various authors have mentioned other predator birds, too: yellow bunting (*Emberiza citrinella* L.) and sparrows (*Passer domesticus*. L. and *Passer montanus* L.) [5].

The earwig *Forficula auricularia* L. (Ord. Dermaptera, Fam. Forficulidae) is mentioned as a pupal predator by many authors. We observed this earwig near the gypsy moth pupae. It did not consume live pupae, only dead pupae, being a necrophagous species.

ADULT PREDATORS

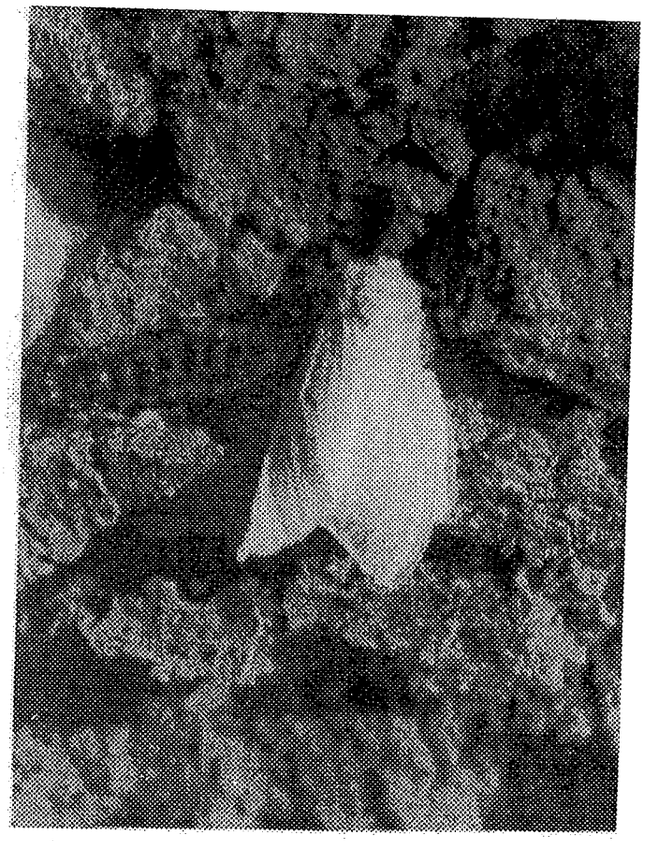

Fig. 6. – A gypsy moth female attacked by a *Silpha carinata* larva.

The gypsy moth females with their egg filled abdomen are hardly mobile, therefore they can be attacked by *Calosoma sycophanta* larvae (Fig .5) and adults and by *Silpha carinata* larvae (Fig. 6), too. We made these observations in the Frasinu wood.

The predator larva or adult approaches the gypsy moth female and usually paralyses it, destroying its thoracic ganglions. Sometimes *Calosoma sycophanta* begins to consume the egg filled abdomen of the gypsy moth female, without paralysing its prey. When the gypsy moth female begins to deposit eggs, it

flaps its wings and sometimes it can even throw the heaving rather predator larva to the ground.

Thus we present the phenomenon af predatism on gypsy moth adults for the first time in science.

CONCLUSIONS

1. We presented seven entomophagous species in the predator complex of the gypsy moth: oophagous predators: *Dermestes erichsoni* (larvae and adults), *Dermestes lardarius* (larvae), *Calosoma sycophanta* (larvae) and an unidentified Heteroptera species (adults); larval predators: *Calosoma sycophanta* (larvae and adults), *Calosoma inquisitor* (larvae) and *Silpha carinata* (adults); pupal predators: *Calosoma sycophanta* (larvae) and jackdaw (*Coleus monedula*); adult predators: *Calosoma sycophanta* (larvae and adults) and *Silpha carinata* (larvae);

2. The presence of the oophagous predators varies from year to year. In some years they are not present but in other years they are very numerous and can contribute to the considerable decrease of the defoliator populations;

3. The most efficient oophagous predators were two dermestid species: *Dermestes erichsoni* and *Dermestes lardarius*. The former species is frequent in all investigated woods and the latter only in Dolj and Gorj counties. *Dermestes erichsoni* could be used in the integrated control projects of the gypsy moth populations;

4. *Calosoma sycophanta* is a predator of all immature stages as well as of adult females of the gypsy moth. We mention the phenomenon of predatism on gypsy moth females with their egg filled abdomen, for the first time in science.

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EVOLUTION TRENDS OF THE DANUBE DELTA LACUSTRIAN ZOOPLANKTON UNDER EUTROPHICATION IMPACT

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Eutrophication increase during the period 1975-1987 resulted in important changes affecting both structure and functionality of the Danube Delta lacustrian zooplankton: the range of taxa suffered a 53% reduction, numerical density increased 4.7 times, biomass increased 6.9 times, productivity being also increased 4.8 times, while the rate of biomass recycling was reduced with 14.52%. A slight trend of impact reduction was noted between 1991-1994.

The Danube river is characterized by one of the biggest and most complex deltas. Its area totalises 4152 km² of which 82% are located on the Romanian territory. Starting with 1991 the Romanian sector of the Danube Delta together with the Razelm lacustrian system of lagoonal origin was declared as a Biosphere Reservation.

From an ecological point of view the Danube Delta represents an aggregate of aquatic and terrestrial ecosystems showing different stages of sequential development which interpenetrate within various ecotone types. Complexity of the relations acting within this aggregate constitutes one of the factors responsible for the biodiversity enhancing, meantime, the vulnerability of deltaic system to different impacts.

Lacustrian system is spread on approximatively 9% of the deltaic area including nearly 500 ecosystems. The areas of the lakes range between 1-1400 ha, their depths ranging between 1.2-3.5 m.

Complex, anthropic factors acting within the entire river basin generated since 1970 successive alterations of the Danube Delta lacustrian ecosystems. As a result, all the stages ranging between mesotrophy and hypertrophy developed rather rapidly within a period of 10-12 years, a relatively short time interval as compared to the natural evolution of such ecosystems. With primary producers the newly created conditions favoured the development of components supporting higher rates of nutrient recycling. As a result a high proliferation of planktonic algae was noted since 1981 which induced the "water blooming", phenomenon determining a dramatic reduction – sometimes even a total elimination – of the submerged macrophytes which used to represent the main primary producer. Maximum effect of the impact was recorded in the period 1981–1984, while the smallest was noted between 1993-1994. If before the impact, the phytoplankton biomass rarely exceeded 5 mg fresh weight/l regarded as a "blooming threshold" (1) much higher values were noted afterwards some of these exceeding 20-30 times the above mentioned limit. From a structural point of view, dominance of Bacillariopyceae

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group was replaced by that of Cyanophyceae algae. Considering algae dimensions the proportions of big-sized algae increased (2). This evolution of primary producers resulted in corresponding mutations influencing the dynamics of planktonic consumers. The impact affected mainly the ecosystems showing the first stage of sequential ecological development decreasing subsequently in stages II and III. Nevertheless it is worth mentioning that important time and space variations of impact intensity were noted as a function of hydrologic factor.

MATERIAL AND METHOD

The researches regarding zooplankton evolution as a function of time and space sequence of the two types of primary producers were conducted in the period 1975-1994 on a number of 12 lacustrian ecosystems showing stage I of sequential ecologic development, i. e. Roşu, Roşuleţ, Porcu, Puiu, Iacub, Isac, Uzlina, Merhei, Matiţa, Babina, Bogdaproste and Băclăneşti.

RESULTS AND DISCUSSIONS

Successive increases in ecosystems trophic stage resulted in important changes affecting structure of nutrient resources of planktonic consumers (decrease in abundance of nanophytoplankton and increase of detritobacterian aggregates). Subsequently these changes result in significant mutations of zooplankton trophic structure (increase in abundance of microconsumers and decrease in macroconsumers). The trophic relations represent the main mechanism of ecosystem self-control function. As a consequence the changes occurring in trophic relations of planktonic subsystem significantly affect zooplankton evolution in structure and function.

Under these circumstances analysis of zooplankton evolution during the period 1975–1987 revealed exceedingly important changes. With ecosystems where the dominance of macrophyte type primary producers continued to be maintained for a while, zooplankton was characterized by a large spectrum totalizing 454 components. Change of trophic relations as well as decrease in ambiental heterogeneity resulted in structural changes evident at the level of secondary consumers which finally resulted in a reduction, exceeding 53%, of taxonomic range, during the above mentioned period (Fig. la). This reduction affected mainly accidental and accessory forms and to a lesser extent constant components. Consequently the ratio of constant forms showed an increase along the process of sequential development from the ecosystems with macrophyte type primary producers (11.41%) to those based on planktonic type primary producers (20.19%) (Fig. 2).

Concurrently numerical density increased 4.7 times, i.e. from 232 to 1094 components/l (Fig. 1b), biomass increased 6.9 times, i.e. from 114 to 790 µg dry matter/l (Fig. lc), productivity being also increased 4.8 times, i.e. from 19.6 to 93.3 mg/1/24h (Fig. 1d). An increasing trend was also noted in the evolution of the ratio of numerically and gravimetrically dominant elements as well as of those having a determining role in productivity, the corresponding figures being the following: 16.74-24.88%, 16.74-20.19% and 11.67-15.96%, respectively (Fig. 2). In the process of functional evolution of zooplankton, the dynamics of biomass

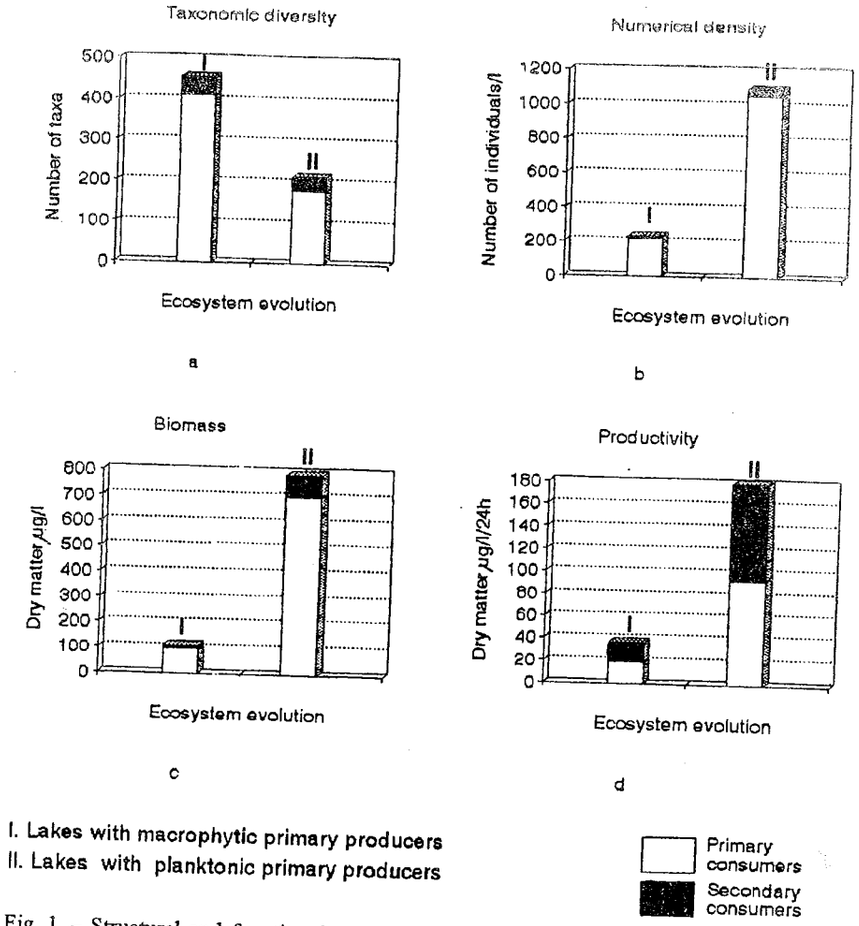

Fig. 1. – Structural and functional parameters of zooplankton in lacustrian ecosystems from Danube Delta with primary producers of macrophytic type (I) and in those with primary producers of planktonic type (II).

recycling rate (B/P/24h) also showed increases, a phenomenon illustrated by a 14.52% reduction of biomass recycling period (i.e. from 9.92 to 8.48 days) (Fig. 3). Under the conditions of the ascending trend of ecosystem trophic state dynamics this functional adaptation represents an important way of improving the extent of using nutritive resources under the new ecological conditions (3).

An eutrophication reduction trend was noted since 1990 in the Romanian sector of the Danube's waters as well as in the Danube's affluents. Consequently in the subsequent period, i.e. 1991-1993 "water blooming" also showed a slight decreasing trend. In 1994 under a favourable hydrological regime a partial recovery of submerse macrophytes occurred while phytoplankton evidenced the lowest values since the impact period (1981–1994). All these improvements of environment and trophic relations affect, to a certain extent, the functional evolution of zooplankton.

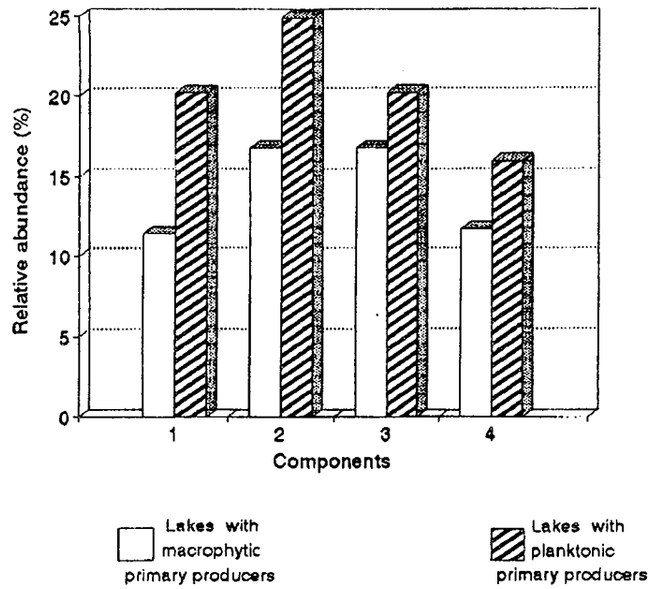

Fig. 2. – Relative abundance (%) of constant elements (1), numerical dominants (2), gravimetrical (3) and productive (4) of zooplankton in ecosystems with macrophytic primary producers and with planktonic primary producers.

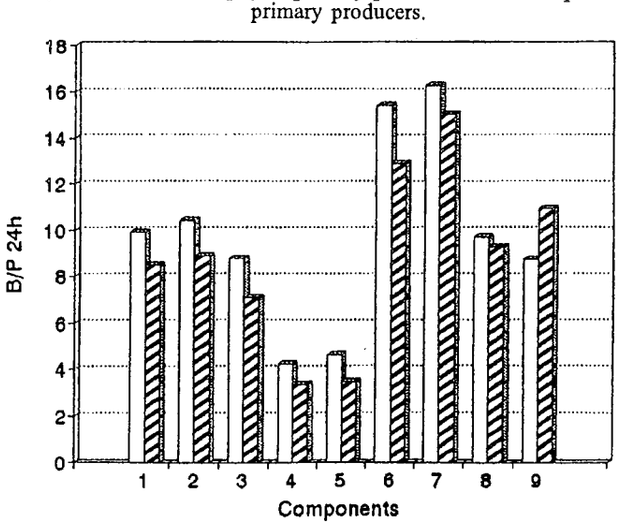

Fig. 3. – Zooplanktonic biomass turnover (B/P/24h) in lacustrian ecosystems with macrophytic primary producers (I) and with planktonic primary producers (II): 1. total zooplankton; 2. total primary consumers; 3. total secondary consumers; 4. rotifers primary consumers; 5. rotifers secondary consumers; 6. copepods primary consumers; 7. copepods secondary consumers; 8. cladocers primary consumers; 9. cladocers secondary consumers.

Naturally, as compared to the situation existing previously to eutrophication impact, the differences are still evident. In the Roşu lake, for example, taxonomic diversity represented in 1994 only 39.13% from that existing in 1975, the values of numerical density, biomass and productivity being 4.6, 10.9 and 10.1 times higher, respectively. Considering the increasing concerns for protection and ecological restoration of the Danube Delta this situation could still be improved.

CONCLUSIONS

– Complex anthropic factor generated since 1970 successive alterations of the Danube Delta lacustrian ecosystems. As a result, all the stages ranging between mesotrophy and hypertrophy developed rather rapidly within a period of 10-12 years.

– Successive increases in ecosystems trophic stage resulted in important changes affecting structure of nutrient resources of planktonic consumers (decrease in abundance of nanophytoplankton and increase of detrito-bacterian aggregates).

– Subsequently these changes result in significant mutations of zooplankton trophic structure (increase in abundance of microconsumers and decrease in macroconsumers).

– Under these circumstances, analysis of zooplankton evolution during the period 1975-1987 revealed exceedingly important changes: the range of taxa suffered a 53% reduction (454→278 components); the ratio of constant forms showed an increase (11.46→20.19%); concurrently numerical density increased 4.7 times (232→1094 components/1), biomass increased 6.9 times (114→790 µg/1 dry matter/1), productivity being also increased 4.8 times (19.6→93.3 µg/1) and biomass recycling rate (B/P/24h) diminished by 14.52% (9.92→8.42 days); an increasing trend was also noted in the evolution of the ratio of numerically and gravimetrically dominant elements (16.74→24.88%, respectively 16.74→20.19%) as well as those having a determining role in productivity (11. 67→15.96).

– An eutrophication reduction trend was noted since 1990 in Danube Delta; thus, in the period 1991-1993 "water blooming" showed a slight decreasing trend; in 1994 under a favourable hydrological regime, a partial recovery of submerse macrophytes occurred, while phytoplankton evidenced the lowest values since the impact period (1981–1994).

– All these improvements of environment and trophic relations affect, to a certain extent, the structural and functional evolution of zooplankton. Naturally, as compared to the situation existing previously to the eutrophication impact, the differences are still evident.

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