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# REVUE ROUMAINE DE BIOLOGIE

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REV. ROUM. BIOL.– BIOL. ANIM., TOME 42, N° 2, P. 85–186, BUCAREST, 1996

# THE DESCRIPTION OF *SPINOSAPSEUDES* N.G., AND AMENDED DIAGNOSES OF TWO GENERA OF TANAIDACEA (CRUSTACEA)

MODEST GUȚU

La nouveau genre *Spinosapseudes* est décrit avec, comme espèce type, *Apseudes setosus* Lang, 1968.
L'espèce *Apseudes diversus* Lang, 1968 este considérée comme appartenant au genre *Atlantapseudes* Băcescu, 1978; par conséquent sa diagnose est amendée et on donne la clé des espèces connues. De même, la diagnose du genre *Carpoapseudes* Lang, 1968 est amendée; *Apseudes austroafricanus* Barnard, 1940, est encadrée dans ce genre et est présentée la clé d'identification des espèces du genre.
On donne un errata de la diagnose du genre *Paradoxapseudes* Guțu, 1991.

While in the latter years remarkable improvements were brought to the systematics of the recent Apseudomorpha, in general, there still persist enough errors regarding both the classification of some morphologically particular species into the wrong genus, and the arbitrary inclusion of some genera into higher taxa.

Thus, in the family Apseudidae, among the wrongly included species into the genus *Apseudes* (subfamily Apseudinae) are *A. diversus,A. setosus* described both by Lang (1968) and *Apseudes austroafricanus* Barnard, 1940.

Another case is that of the species *Carpoapseudes curticarpus* Băcescu, 1982 (subfamily Leviapseudinae) which does not fit into the diagnosis of the genus since the carpus is shorter than the merus.

Regarding the species *Apseudes setosus* I think that here I have a new genus. On analysing the morphology of pereopods II of this species one finds a close similarity with those of the genus *Carpoapseudes* Lang, 1968, except that in *A. setosus* the carpus is shorter than the merus. But Lang (1968, pp 43 and 62), both in his description of *A. setosus* and in the diagnosis of the genus *Carpoapseudes*, emphasizes that pereopod II is not adapted for digging as in the other species of the genus *Apseudes* (and even in those of the family Apseudidae) in which pereopods II are adapted for digging. Because of the presence of a spiniform apophysis on the coxa of pereopod II the species must be considered to belong to the family Apseudidae, and because of the shape of the inner caudo-distal seta of the maxillipedal endite, the new genus should be included in the subfamily Apseudinae, appearing as an "intermediate" genus between the Apseudinae and the *Carpoapseudes*, similarly as other species or genera of the same subfamily Apseudinae recall the genus *Leviapseudes*, once more confirming that all these genera should be placed in the same family.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 41, N° 2, P. 87–93, BUCAREST, 1996

## Family APSEUDIDAE Leach, 1814

## Subfamily Apseudinae Leach, 1814

### *Spinosapseudes* n.g.

(Figs 1–2)

*Type species*: *Apseudes setosus* Lang, 1968

*Diagnosis*: Body relatively cylindrical, with lateral spiniform prolongations of carapace (on the first half), pereonites and pleonites; ocular lobes well developed, extended as a spiniform process, without visual elements (Fig. 1A). Peduncle of antenna five-articulated; second joint longer than the fourth or fifth; squama present (Fig. 1B). Mandible with palp, three-articulated. Maxillule with two-articulated palp. Inner caudo-distal seta of maxillipedal endite, normal (not leaf-like). Exopodite of cheliped and pereopod II present. Pereopod II stronger than the following ones, but not adapted to digging; coxa with spiniform apophysis; merus, carpus and propus with numerous and long setae (on the sternal and tergal borders) and several long spines (Fig. 1C). Pereopods III-VII thin, without spines on the merus, carpus and propus (only short or long setae); coxa provided with small spines (Figs 1 A-E). Pleopods biramous, well developed, in five pairs. Uropods filiform, biramous.

*Sexual dimorphism* consists in the number of joints of the flagella of antennule, antenna and uropods.

*Gender*: masculine.

*Etymology*: of the Latin *spinosus*, "with spines" and *Apseudes*, relating to the big spines of the body, and similarly with named genus.

*Distribution*: Tasmanian Sea.

*Remarks*: *Spinosapseudes* n.g. differs from the other genera of the subfamily Apseudinae by the lack of robust spines on the merus, the carpus, and the propus of pereopod II (Fig. 1C) and by the presence on the coxa of pereopods III-VII of one or two acute spiniform processus (Fig. 2 A-E). As mentioned, pereopod II resembles very much those in the genus *Carpoapseudes*.

The description of this new genus brings the number of genera of the subfamily Apseudinae to nine, and that of the family Apseudidae to twelve.

## Genus *Atlantapseudes* Băcescu, 1978

As for the species *Apseudes diversus* Lang, 1968 this should be considered to belong to the genus *Atlantapseudes* Băcescu (1978). The arguments that support this statement are: the male antennule with esthetascs; peduncle of antenna of same type (very long joint two, four, and five); squama reduced in size (similar as in the species *Atlantapseudes lindae* Meyer and Heard, 1989); no exopodite of pereopod II.

The major differences with the other two known species (*Atlantapseudes nigrichela* Băcesu, 1978 and *A. lindae*) consist in the presence of rudimentary uniramous pleopods in the females and of the cheliped with exopodite (in both sexes) in the *Apseudes diversus*.



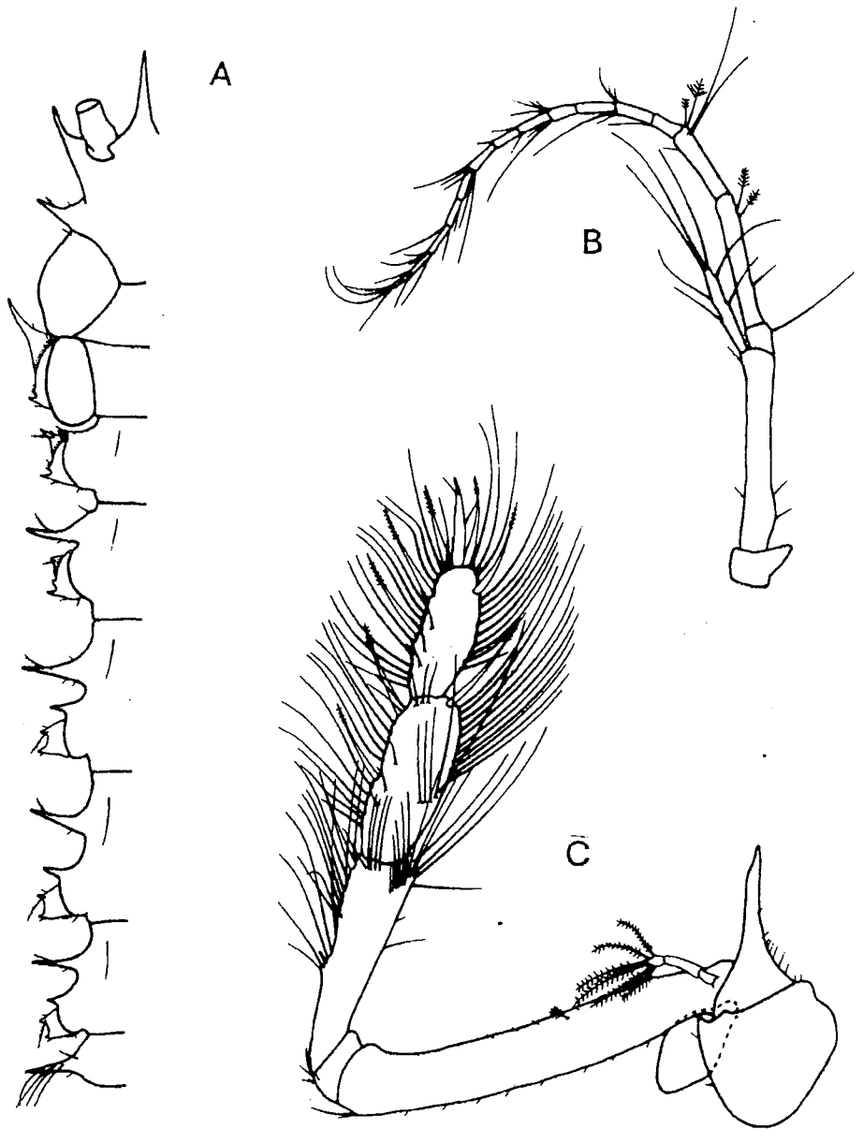


Fig. 1. – *Spinosapseudes setosus* (Lang, 1968), female: A = left side of carapace, pereon and first pleonite; B = antenna; C = pereopod II (after Lang, 1968).

Nevertheless, I believe that *A. diversus* is closer to *Atlantapseudes* than to the genus *Apseudes* or other genera. It is a known fact that other genera, too, have species with or without pleopods, and when they have pleopods these can be differently or numerically developed. The study of the recent Apseudomorpha

reveals that, generally, the antenna is morphologically more constant than the pleopods within any genus, which makes us believe that *A. diversus* belongs to the genus *Atlantapseudes*. However, I do not exclude the alternative of the existence of a separate subgenus within the genus *Atlantapseudes* or even of new genus. When more related species are discovered, this could become a certainty.

According to the aforementioned arguments, the diagnosis of the genus *Atlantapseudes* must be amended, in furtherance of the already amending by Meyer and Heard (1989).

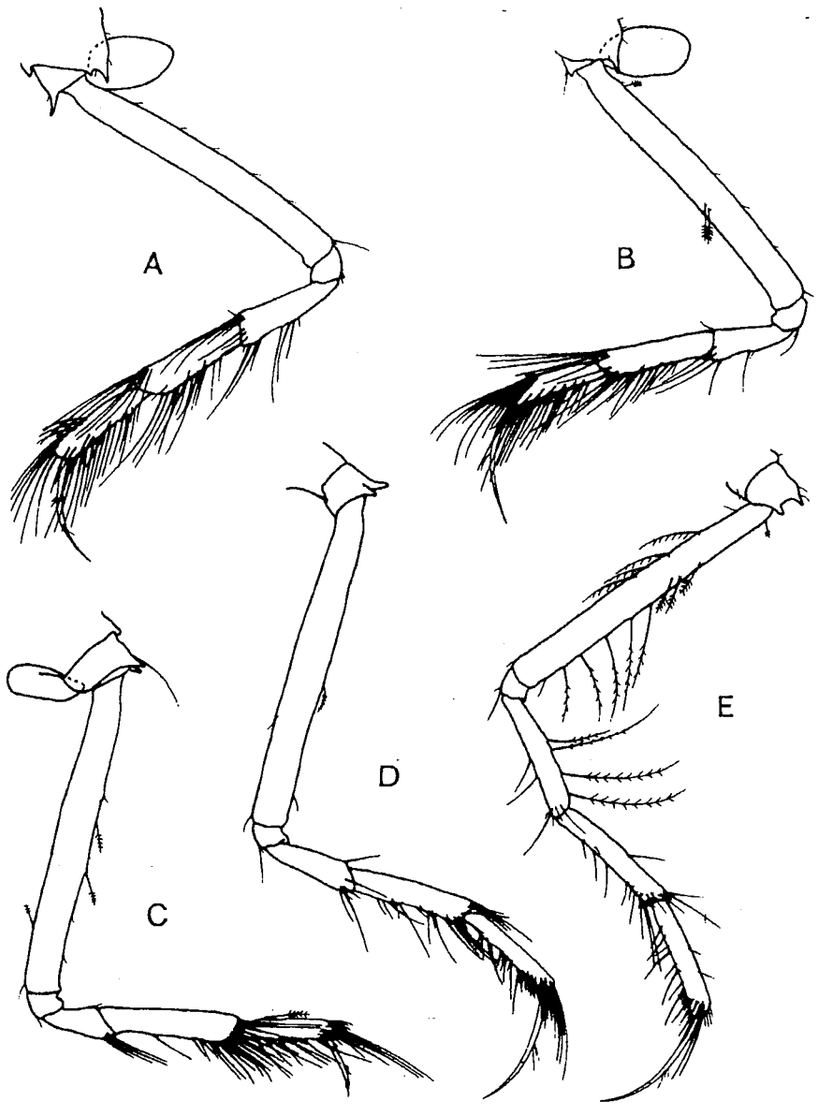


Fig. 2. – *Spinosapseudes setosus* (Lang, 1968), female: A – E = pereopods III–VII (after Lang, 1968).



*Amended diagnosis*: Carapace with ocular lobes well defined, extended as a spiniform prolongation. The free pereonites two-six with or without spiniform processus on the lateral sides. Peduncle of antenna five-articulated; the joints two, four and five very long; squama present, thin, reduced in size or not. Palp of mandible three-articulated. Maxillule with bi-articulated palp. Inner caudo-distal seta of maxillipedal endite, normal (not leaf-like). Cheliped with or without exopodite; pereopod II without exopodite. Pereopod II stronger than the following ones, adapted to digging; coxa extended as a spiniform process. Pereopods III-VII thin. Pleopods absent or present, uni-ramous and reduced numerically in females, and in five pairs, uni- or bi-ramous, reduced in size or not, in males. Uropods filiform, biramous.

*Sexual dimorphism*: The males differ from the females in antennulae, chelipeds and pleopods (when these are present).

Key to the species of the genus *Atlantapseudes*

1 – Cheliped with exopodite; first two pairs of pleopods uniramous . . . . . *A. diversus* (Lang, 1968)
– Cheliped without exopodite; first two pairs of pleopods biramous or absent. . . . . 2
2 – Carapace with lateral spines in first half; pereonites two-six with well-developed lateral spines; antenna with thin and long squama. . . . . *A. nigrichela* Băcescu, 1978
– Carapace without lateral spines in first half; pereonites two-six without lateral spines or only with small prominences; antenna with reduced squama. . . . . *A. lindae* Meyer & Heard, 1989

Subfamily Leviapseudinae Sieg, 1980

Genus *Carpoapseudes* Lang, 1968

The morphology of the species *Carpoapseudes curticarpus* extremely similar to that of the other species of the genus *Carpoapseudes* determined Băcescu (1982) to include it also in this genus, although the main character of the latter as established by Lang (1968) is not taken into consideration. The question is that the carpus of pereopod II compared to the merus is in the genus *Carpoapseudes* longer, but shorter in the species *C.curticarpus*; this distinction should be sufficient to create a new genus. On analyzing very objectively the situation, I concluded that, at least for the moment, it was not the case to include *C. curticarpus* into a separate genus. When I decided this I thought that it was not impossible for other resembling species to be discovered in the future but in which the carpus of pereopod II should be of equal length with the merus, with small differences between the males and the females, which obviously would lead to more serious confusion. That is why I believe that the genus *Carpoapseudes* (including *C. curticarpus*) has sufficient other morphological features that can delimit this genus from *Leviapseudes* which it joins in the subfamily Leviapseudinae, and likewise the other genera of the family

Apseudidae. I consider the general morphology of pereopod II blended with the inner caudo-distal seta of the endite of the maxilliped (leaf-like), and also the shape and morphology of the body, antennule, antenna, pleopods etc.

At the same time, in my opinion, the species *Apseudes austroaficanus* Barnard, 1940, belongs to the genus *Carpoapseudes*.

Consequently, I altered the diagnosis of the genus *Carpoapseudes* as formulated by Lang (1968) so as to allow the inclusion of the species *C. curticarpus* and, eventually, of other species as well.

*Amended diagnosis*: The body relatively cylindrical, with lateral processus on the pereonites three-five. Carapace triangular, without spiniform apophysis; ocular lobes present, extended as a spiniform prolongation. The free pereonites four and five longest; last pereonite short and trapezoidal. Pleotelson cylindrical and long. Antenna with five-articulated peduncle; squama present, well developed. Mandible with three-articulated palp. Maxillule with bi-articulated palp. Inner caudo-distal seta of maxillipedal endite transformed into a leaf-like spine. Cheliped and pereopod II with exopodite. Pereopod II stronger than the following ones; coxa extended as a spiniform prolongation; basis long and cylindrical; merus, carpus, and propus with numerous setae and thin spines; usually carpus longer than the merus (only occasionally shorter). Pereopods III-VII thin and long. Pleopods well developed, in five pairs. Uropods filiform, biramous. Oostegites in five pairs.

*Sexual dimorphism* is present on the cheliped and antennule, but not very accentuated.

Key to the species of the genus *Carpoapseudes*

1 – Carpus of pereopod II shorter than merus. . . . . *C. curticarpus* Băcescu, 1989
– Carpus of pereopod II longer than merus . . . . . 2
2 – Carapace without acute rostrum (rounded anteriorly). . . . . *C. rotundirostris* Kud.-Past., 1989
– Carapace with acute rostrum (long or not). . . . . 3
3 – Exopodite of cheliped longer than half of the basis. . . . . *C. menziesi* Guțu, 1975
– Exopodite of cheliped shorter than half of the basis. . . . . 4
4 – Antenna with six-articulated flagellum . . . . . *C. austroafricanus* (Barnard, 1940)
– Antenna with at least ten-articulated flagellum . . . . . 5
5 – Rostrum very short; flagellum of antenna with ten long joints. . . . . *C. romanae* Băcescu, 1978
– Rostrum long; flagellum of antenna with more than ten joints. . . . . 6
6 – Basis of cheliped with 3–4 tuberculiform processus on the tergal board, in the proximal end. . . . . *C. laubieri* Băcescu, 1982
– Basis of cheliped smooth or only with one tubercule in the proximal end. . . . . 7
7 – Outer flagellum of antennule with more than 38 joints. . . . . *C. longissimus* Lang, 1968



– Outer flagellum of antennule with less than 35 joints. . . . . 8
8 – Basis of pereopod II with a spiniform tubercule (or tooth) near exopodite . . . 9
– Basis of pereopod II without a spiniform tubercule (or tooth) near exopodite. . . . . 11
9 – Propus of cheliped without an excavation in the first half of sternal side. . . . . *C. oculicornutus* Lang, 1968
– Propus of cheliped with an exavation in the first half of sternal side. . . 10
10– Inner flagellum of antennule with seven joints. . . . . *C. kudinovae* Băcescu, 1981
– Inner flagellum of antennule with more than ten joints. . . . . *C. simplicirostris* (Norm. & Stebb., 1886)
11– Propus of cheliped with an excavation in the first half of sternal side. . . . . *C. auritochelis* Kud.-Past., 1975
– Propus of cheliped without an excavation in the first half of sternal side. . 12
12– Inner flagellum of antennule with 7–8 joints. . . . . *C. serratispinosus* Lang, 1968
– Inner flagellum of antennule with 11 joints. . . . *C. bacescui* Guțu, 1972

*Remarks*: Despite all our efforts, it might be that the very brief description of some species had an unfavourable influence on our conclusion with regard to some of the features used in the present identification key.

Family TANAPSEUDIDAE Băcescu, 1978

Genus *Paradoxapseudes* Guțu, 1991

By a most regrettable error (not observed in the prints), in the paper in which I described the genus *Paradoxapseudes*, a whole line from the manuscript was omitted which led to a most confusing statement in the diagnosis ("Antenna with five-articulated palp"; Guțu, 1991, p. 349). Thus, exactly the main morphological feature that delimits this genus from *Tanapseudes* Băcescu (1978) was erroneously eliminated from the text. Therefore, instead of the above quotation, in the diagnosis of the genus *Paradoxapseudes* one should read: "Antenna with five-articulated peduncle; squama present. Mandible with three-articulated palp".

*Acknowledgements*. I express my sincere thanks to Mrs. Roxana Georgescu for helping with the translation and Miss Adriana Onicel who inked the drawings and typed the text.

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# TROIS ESPÈCES NOUVELLES DU GENRE AFROTROPICAL *XANTHOPTERISCA* ROHDENDORF (DIPTERA, SARCOPHAGIDAE)

ANDY Z. LEHRER

Three new species: *Xanthopterisca aheria* n. sp., *Xanthopterisca mazaliana* n. sp. and *Xanthopterisca nihbadella* n. sp. from South Africa are described.
Dans les collections diptérologiques du Natal Muséum, Pietermaritzburg, nous avons trouvé encore trois espèces nouvelles du genre *Xanthopterisca* ROHDENDORF de l'Afrique de Sud, à savoir: *X. aheria* n.sp., *X. mazaliana* n. sp. et *X. nihbadella* n. sp. Ainsi, le genre *Xanthopterisca* Rohdendorf totalise 9 taxons jusqu'à présent.

## DESCRIPTION DES NOUVELLES ESPÈCES

### 1. **Xanthopterisca aheria** sp. n.

MÂLE

*Tête*. Noire, avec le tomentum argenté. Front, vu du dessus et au lieu le plus étroit, mesure 1/2 de la largeur d'un œil. La bande frontale noire est deux fois plus large qu'une parafrontalie. Les antennes sont noires à légère nuance brunâtre; le troisième article est 1,5 fois plus long que le deuxième. Arista est noire brunâtre, avec des poils moyens sur les deux parties. La trompe est relativement plus longue et plus mince, d'un noir brunâtre et avec les ventouses assez petites; les palpes noirs brunâtres. Le péristome mesure presque 2/5 du grand diamètre oculaire.

*Chétotaxie de la tête*. Les macrochètes verticaux internes sont longs, forts et rétroclines; les macrochètes verticaux externes indistincts; les ocellaires proclines fins et longs; les préverticaux rétroclines sont plus développés que les précédents; les macrochètes frontaux sont au nombre de 11 paires; les parafaciaux fins et courts; les petites vibrisses montent un peu sur les bordures faciales; on voit seulement 2 postocellaires et postverticaux sur chaque côté de l'occiput; les microchètes occipitaux disposés sur deux rangs. Péristome couvert de poils noirs; la partie postérieure de la tête a des poils blancs, relativement plus rares.

*Thorax*. Noir à tomentum argenté, trois bandes médio-dorsales longitudinales noires larges et deux bandes latérales étroites. Propleures glabres; prosternum poilu. Les stigmates antérieurs noirâtres; les stigmates postérieurs bruns rougeâtres. Les pattes sont noires, seulement les tibias ont une légère nuance brunâtre; les fémurs médians n'ont pas un cténidium typique..

*Chétotaxie du thorax*. ac = 0 + 1, dc = 4 + 4 (les présuturaux et les premiers deux postsuturaux sont fins et courts), ia = 0 + 2, prs = 1, sa = 3, h = 3, ph = 1, n = 4, pa = 2, sc = 4 + 0 (les basaux et les apicaux sont très fins et piliformes), pp = 1 (plus un macrochète), pst = 1 (plus un poil), st = 1:1:1.

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*Ailes*. Transparentes. Épaulette noire; basicosta et costagium jaunes. La nervure $r_1$ est glabre. La nervure $r_{4+5}$ est pourvue de cils jusqu'à la moitié de la distance entre son origine et r – m. Cubitulus est courbé en angle obtus et prolongé d'un pli. L'épine costale manque. Les écailles blanches à nuance légère jaunâtre; les balanciers bruns avec le capitulum jaune.

*Chétotaxie des tibias*. Les tibias antérieurs ont 3 ad proximaux petits et 1 pv. Les tibias médians sont pourvus de 2 ad, 0 av, 2 pd, 1 pv et une longue pilosité sur les parties postéro-ventrales. Les tibias postérieurs ont 2 ad, 1 av, 2 pd et une longue pilosité sur les parties antéro- et postéro-ventrales.

*Abdomen*. Noir, avec tomentum argenté et dessins en damiers. La formule chétotaxique: 0 + 0 + 2 + série. Le tergite génital est noir luisant, avec la marge postérieure tachée de jaune-orange et quelques poils marginaux plus longs. Le tergite anal orange.

*Armature génitale:* fig. 1. Le sternite V (A) a des brosses et une forme caractéristique pour le genre *Xanthopterisca* Rohd.; les condyles sont grands. Cerques (B) relativement un peu plus longs et plus étroits, légèrement courbés et avec le sommet un peu arrondi; les paralobes sont allongés, étroits et avec les sommets arrondis. Distiphallus (C) petit. Theca presque aussi large que la partie basale du paraphallus. La membrane est courte, transparente et peu pliée. Les lobes membranaux sont bien développés, orientés obliquement en bas, très sclérifiés et

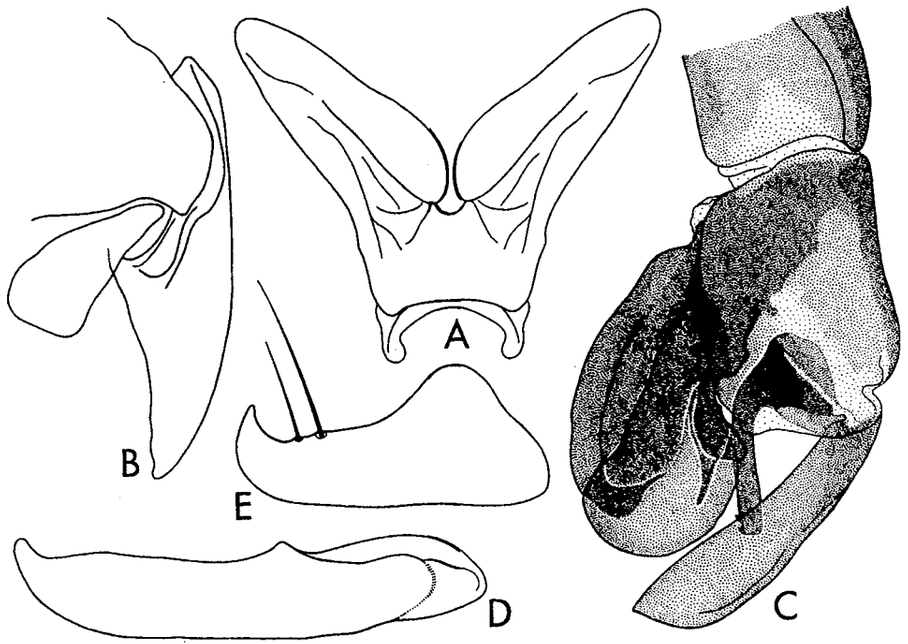


Fig. 1. – Armature génitale mâle de *Xanthopterisca aheria* n. sp. A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.



pigmentés; les lobes externes ont une forme de raquette, largement arrondis à la partie antéro-terminale; ceux internes sont allongés, légèrement courbés sur la marge supérieure et presque aussi longs que les précédents. La partie basale du paraphallus est prolongée avec les lobes paraphalliques assez petits et partiellement visibles par transparence. La partie apicale du paraphallus est formée de deux apophyses latérales assez larges et aiguës au sommet; elles arrivent jusqu'au bout des lobes membranaux. Les styles sont tubulaires, mais très petits, étroits, ne dépassant les apophyses latérales et avec deux dents apicales courtes. Le lobe hypophallique est court et visible seulement par transparence; il ne dépasse pas la marge inférieure des lobes membranaux externes. Les prégonites (D) sont plus longs que les postgonites (E); les premiers sont droits et avec un sommet très court; les seconds ont la forme d'un crochet à la base large et deux macrochètes superterminaux.

*Longueur du corps:* 10 mm.

FEMELLE. Inconnue.

*Matériel étudié*. 1 ♂ holotype, ayant l'étiquette: SOUTH AFRICA, Cape Prov., 5 km N Nieuwondville, 5.IX.1981, 3119AC, J. Londt, L Schoeman and B. Stuckenberg; W Mountain Karoo; 1 ♂ paratype à l'étiquette: «STH AFRICA: Cape Prov., Tradouw Pass, Swellendam Dist. w. Cape, 26.III.1986, Stuckenberg»; 1 ♂ paratype, avec l'étiquette: «STH AFRICA: Cape Prov., 10 km SE Tulbagh, 3319 AC, 30.VIII.1981, J. Londt, L. Scheoman and B. Stukenberg, Macchia near stream»; 1 ♂ paratype à l'étiquette: «STH AFRICA: Cape Prov., 8 km N Kamieskroon, 30°08'00"S: 17°55'30"E, 31.VIII.1989, 900 m, J. Londt, B. Stuckenberg & P. Croeser, Rocky E slope/road cutting»; 1 ♂ paratype à l'étiquette: «STH AFRICA: Cape Prov. Stuers Pass 22 km NE of Garies, 3018AC, 6.IX.1982, J. Londt, B. Stuckenberg, Stream edge & rocky slopes».

## 2. **Xanthopterisca mazaliana** sp. n.

MÂLE

*Tête*. Noire, tachée de brun sur la marge antérieure des parafacialies et sur le vibrissarium avec ses branches faciale et suboculaire, et couverte d'un tomentum argenté. Front étroit; vu du dessus et au lieu le plus étroit, il mesure 1/3 de la largeur d'un œil. La bande frontale noire est de 2,5-3 fois plus large qu'une parafrontalie. Antennes noires, courtes; le troisième article est de 1,5 fois plus long que le deuxième. Arista brune, assez courte et avec des poils moyens sur les deux parties. La trompe est noire; les palpes sont bruns et très courbés en haut. Péristome mesure 1/3 du grand diamètre oculaire.

*Chétotaxie de la tête*. Les macrochètes verticaux internes sont longs, forts et rétroclines; les macrochètes verticaux externes sont distingués et 1/2 des précédents; les ocellaires proclines sont plus fins que les préverticaux rétroclines, mais assez longs; les frontaux sont au nombre de 10-11 paires, relativement fins et avec la dernière paire rétrocline; les macrochètes parafaciaux sont longs et fins; les petites vibrisses montent un peu sur les bordures faciales; on voit 1 postocellaire et 1

postvertical sur chaque côté de l'occiput; les microchètes occipitaux sont disposés sur deux rangs. Le péristome est couvert de poils noirs; la partie postérieure de la tête a de poils blancs.

*Thorax*. Noir et avec tomentum argenté, trois bandes médiodorsales longitudinales noires et larges, et deux bandes latérales étroites. Propleures et prosternum glabres. Les stigmates antérieurs sont noir-brunâtres; ceux postérieurs brun-rougeâtre. Les pattes sont brunes; les fémurs médians sans cténidium typique.

*Chétotaxie du thorax*. ac = 0 + 0, dc = 3 + 3, ia = 1 + 2, prs = 1, sa = 3, h = 3, ph = 1, n = 4, pa = 2, sc = 3 + 1 (ap et d sont très fins), pp = 1 (plus 1 macrochète), pst = 1 (plus un poil), st = 1 : 1 : 1.

*Ailes*. Transparentes. Epaulette noire; basicosta et costagium sont jaunes. La nervure $r_1$ est glabre. La nervure $r_{4+5}$ pourvue de cils sur 1/2 de la distance entre son origine et r–m. Cubitulus est courbé en angle droit et prolongé d'un pli. L'épine costale très petite. Les écailles sont blanches; les balanciers jaune-brunâtres.

*Chétotaxie des tibias*. Les tibias antérieurs ont 3 ad proximaux et 1 pv. Les tibias médians sont pourvus de 2 ad, 1 av, 2 pd et 1 pv. Les tibias postérieurs ont 2 ad, 1 av, 2 pd et une longue pilosité sur les parties antéro- et postéro-ventrales.

*Abdomen*. Noir, avec le tomentum argenté et les dessins en damiers. La formule chétotaxique;: 0 + 0 + (2 + 2 + 2) + série. Le tergite génital et brun-rougeâtre, luisant et sans tomentum dorsal ou macrochètes marginaux. Le tergite anal orange.

*Armature génitale*: fig. 2. Le sternite V(A) bien développé et pourvu de brosses; sa partie basale est relativement longue et large, avec condyles très petits; les lames latérales sont longues et avec les sommets arrondis. Les cerques (B) plus

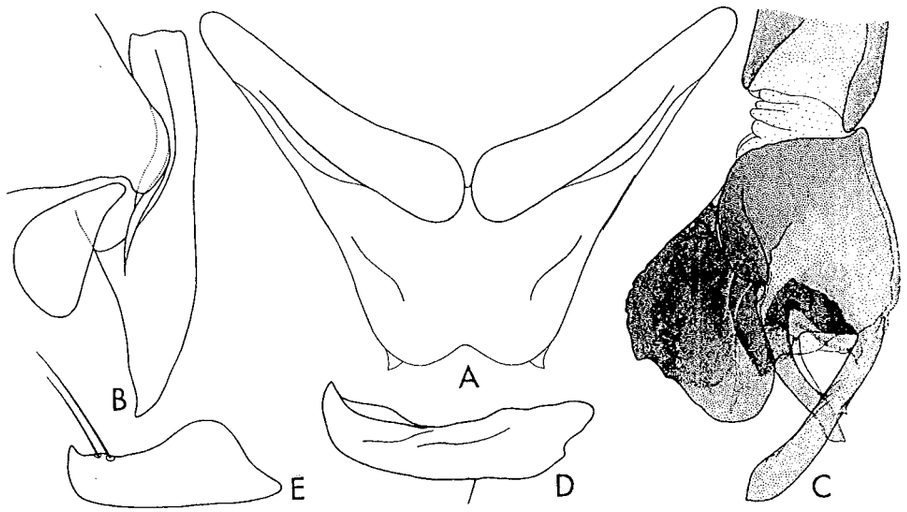


Fig. 2. – Armature génitale mâle de *Xanthopterisca mazaliana* n. sp. A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.



longs et assez larges, triangulaires, légèrement courbés dorsalement et ayant un sommet court; les paralobes sont larges, arrondis et d'une grandeur habituelle. Distiphallus (C) petit. La partie basale du paraphallus est plus ou moins cvadrangulaire, ayant la marge supérieure droite et prolongée avec les lobes paraphalliques étroits et assez allongés, situés sous les lobes membranaux. La partie apicale du paraphallus est formée de deux apophyses latérales étroites, relativement courtes, orientées obliquement en bas, ayant le sommet un peu aigu et une dent submédiane très petite sur la marge inférieure. La membrane est courte, transparente et avec quelques plis. Les lobes membranaux sont relativement plus courts, mais très élargis dans la région antéro-inférieure; leur marge est légèrement creusée irrégulièrement et plus ou moins ondulée; leur marge inférieure est largement arrondie. Les styles sont courts, minces, courbés en bas et pourvus de 3 dents apicales petites. L'apophyse hypophallique qui s'observe par transparence a un sommet long, mais il ne dépasse pas la marge inférieure des lobes membranaux. Les prégonites (D) sont plus longs que les postgonites (E); les premiers ont une crête apicale courte et un macrochète submédian inférieur petit; les derniers ont la forme d'un crochet, avec le sommet très petit et 2 macrochètes superterminaux longs.

*Longueur du corps*: 8,5 mm.

FAMELLE. Inconnue.

*Matériel étudié*. 1 ♂ holotype de l'AFRIQUE DU SUD, ayant l'étiquette «12 sept. 24, Dr. Brauns, Wellington, Cape Colony».

### 3. **Xanthopterisca nihbadella** sp. n.

MÂLE

*Tête*. Noire et couvert d'un tomentum argenté. La front, vu du dessus et au niveau le plus étroit, mesure 1/2 de la largeur d'un œil. La bande frontale noire est deux fois plus large qu'une parafrontalie. Sur le vertex et à la partie postérieure de chaque parafrontalie il y a, d'habitude, une tache noire luisante, ovale et égale à la longueur du triangle ocellaire. Les antennes noires ont une légère teinte brunâtre; le troisième article est 1,75-2 fois plus long que le deuxième. Arista est noire brunâtre et pourvue de poils moyens sur les deux parties. Trompe et palpes noirs; les derniers ont une nuance brunâtre et l'apex brun. Péristome mesure 2/5 du grand diamètre oculaire.

*Chétotaxie de la tête*. Les macrochètes verticaux internes sont assez forts, longs et rétroclines; les macrochètes verticaux externes sont très fins et peu distincts; les ocellaires fins; les préverticaux rétroclines sont bien développés; les frontaux sont au nombre de 8 paires; les macrochètes parafaciaux assez longs et plus nombreux; les petites vibrisses montent un peu sur les bordures faciales; on voit 1 postocellaire et 1 postvertical sur chaque côté de l'occiput; les microchètes occipitaux sont disposés sur deux rangs. Le péristome a de poils noirs; la partie postérieure de la tête de poils blancs.

*Thorax*. Noir, avec le tomentum argenté à nuance jaunâtre, trois bandes médio-dorsales longitudinales noires et larges, et deux bandes latérales étroites. Propleures glabres; prosternum avec quelques poils. Les stigmates sont jaunes. Les pattes noires ont les tibias d'un brun foncé; les fémurs médians sans cténidium typique.

*Chétotaxie du thorax*. ac = 0 + 0 – 1, dc = 5 + 4 – 5, ia = 1 + 2 – 3, prs = 1, sa = 3, h = 3, ph = 2, n = 4, pa = 2, sc = 3 +1 (ap très fins et croisés), pp = 1 (avec quelques poils), pst = 1, st = 1:1:1.

*Ailes*. Transparentes. Épaulette noire; basicosta et costagium sont jaunes. La nervure $r_1$ est glabre. La nervure $r_{4+5}$ est ciliée sur une distance de 2/3 entre son origine et r – m. Cubitulus est courbé en angle droit et prolongé d'un pli. L'épine costale manque. Les écailles sont blanches; les balanciers d'un jaune brunâtre.

*Chétotaxie des tibias*. Les tibias antérieurs ont 2–3 ad proximaux et 1 pv. Les tibias médians sont pourvus de 2 ad, 1 av, 2 pd et 1 pv. Les tibias postérieurs ont 2 ad, 1 av, 2 pd et une longue pilosité sur les parties antéro- et postéro-ventrales.

*Abdomen*. Noir, avec tomentum argenté et dessins en damiers; mais, il a une bande médiane noire et très large sur les tergites I-IV. La formule chétotaxique: 0 + 0 + 2 + série. Le tergite génital est brun sur la moitié basale et orange sur celle postérieure; il n'a pas de macrochètes marginaux. Le tergite anal est orange.

*Armature génitale:* fig. 3. Le sternite V (A) est petit et pourvu de brosses; sa partie basale est plus étroite, courte et avec les condyles plus développés; les lames latérales sont larges, assez longues et arrondies aux bouts. Les cerques (C) sont petits, triangulaires, légèrement courbés dorsalement et ont un sommet très court; les paralobes sont relativement longs et larges. Distiphallus (C) petit. La partie

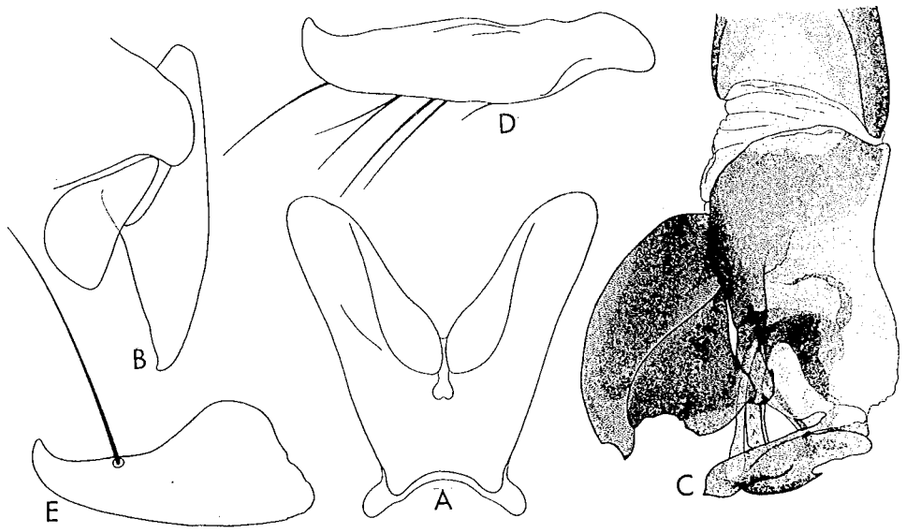


Fig. 3. – Armature génitale mâle de *Xanthopterisca nihbadella* n.sp. A = sternite V; B = cerques et paralobes; C = distiphallus; D = prégonites; E = postgonites.



basale du paraphallus est très développée, longue, plus ou moins cvadrangulaire, avec la marge supérieure presque droite et prolongée avec les lobes paraphalliques étroits, peu allongés et pourvus d'une épine dorsale. La partie apicale du paraphallus est formée de deux apophyses latérales orientées ventralement en angle droit, courtes, avec les sommets un peu aigus et pourvus d'une lame interne qui a une dent postérieure bien visible. La membrane est courte et transparente. Les lobes membranaux sont très pigmentés et sclérifiés; les lobes internes sont très longs, courbés largement en bas, ayant une échancrure subapicale et un sommet un peu courbé; les lobes externes sont plus courts, plus ou moins triangulaires, larges et avec la marge inférieure ayant de petits évidages ondulés et irréguliers. Les styles sont assez minces, courts, ne dépassant pas le milieu des apophyses latérales de la partie apicale du paraphallus et ont 3 dents apicales. L'apophyse hypophallique est très longue, mince, un peu élargie au bout terminal et dépasse le sommet des styles. Les prégonites (D) sont plus longs que les postgonites (E); les premiers ont le sommet un peu courbé, court et pourvus avec quelques macrochètes longs sur la marge inférieure; les seconds ont la forme d'un crochet, avec la base très large et un macrochète subapical très long.

*Longueur du corps*: 6-7,5 mm

FEMELLE. Inconnue.

*Matériel étudié*. 1 ♂, holotype, avec l'étiquette: SOUTH AFRICA: Natal, Ashburton 15 km SE of Pietermaritzburg, Feb. 1977, J.G.H. Londt. Malaise in grassland. 6 ♂♂ paratypes avec les mêmes dates, mais 5 mâles ont été colligés au mois de Janvier.

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# *FILENCHUS CYLINDRICUS* AND *F. MISELLUS* NEW SPECIES OF NEMATODES FROM ROMANIAN FAUNA

IONELA DOBRIN and I. ROŞCA

Morphology and systematics of two plant parasitic nematodes from Romania are studied. Two members of family Tylenchidae: *Filenchus cylindricus* (Thorne and Malek, 1968), Niblack and Bernard, 1985, *F. misellus* (Andrassy, 1958) Geraert and Raski, 1987 are studied and described. They are recorded for the first time from Romania.

Pricina, in 1910, mentioned the presence of *Ditylenchus dipsaci* named *Tylenchus devastatrix* and *T. putrefaciens* (8). Manolache, in 1951, confirmed the presence of *D. dipsaci* quoted by Romaşcu and this author in 1968 studied this nematode in association with *D. destructor*(10). At the same time Popovici mentioned the presence of *Pratylenchus penetrans* in Cluj and six years later she found *Psilenchus hilarulus* in natural grassland, *Anguina tritici* and *Heterodera schachtii* were mentioned by Rădulescu in 1937(7). Later on a number of papers about attack and control were published and were registered by Romaşcu in 1973 (10): Bontea (1937), Rădulescu and Gruiţa (1941), Bretan and Bretan (1943), Manolache (1947-1955), Beratlief (1963), Romaşcu (1965-1969), Motoi (1967) and Brad (1968). The first reference about *Meloidogyne* in Romania was in 1970, when *M. incognita* was identified (Kop and White, pers. comm.).

*M. arenaria* was found in 1971 in cultivated soil with carrots and in 1973, *M. hapla* was found in *Zantedeschia aethiopia.*

Beginning with 1984, when in Romania *Globodera rostochiensis* was discovered, most of the investigations were pointed to this particular species (Rojancovschi, pers. comm.).

## MATERIAL AND METHOD

The soil samples were collected in September 1993 from cultivated soil with maize from Fundulea, a locality situated 35 km from Bucharest. Other soil samples from Bucharest were taken from the field, previously cultivated with potatoes, parsley, onion, garlic and cabbage.

The samples were taken nearly roots of plant, at 0-20 cm depth. Each sample of 1 kg was mixed and 150 g was fixed with hot formalin 4% (at 70°C). The nematodes were extracted from fixed soil samples by the centrifugal-flotation method (6).

Permanent slides were mounted according to Cobb using the paraffin-ring method of the Maeseneer & D'Herde, methods described by De Grisse (3). A drawing tube attached to a light microscope Wild M 12 was used to make

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measurements and drawings. The higher magnification (50× ; 100×) was used for all measurements:

measurements were taken with digital ruler pen;

in the description of the species, the De Man's formulae of body ratios for nematodes measurements are used.

The abbreviations used in tables and descriptions are:

*n* = number of specimens;

*L* = total body length in μm or mm;

a = body length/maximum body widht;

b = body length/oesophageal length;

c = body length/tail length;

c'= tail length/anal body width;

V = distance from anterior end to vulva ×100/total body length;

V' = vulva length ×100/distance from the head to the anus;

VA/T as % = vulva anus distance as a percentage of tail length;

MB = distance from anterior end to the median bulb of the oesophagus ×100/ oesophagus length;

G = total length of female anterior branch as % of body length;

G' = total length of female posterior branch as % of body length;

stylet = length of stylet in μm;

LL = lateral field lines;

SEM = Scanning Electron Microscope;

LM = Light microscope.

The nematodes used for SEM observations were fixed in a 4% formaldehyde solution and mounted on slides in dehydrated glycerine following Seinhorst's rapid method (7).

The model JSM-840 scanning microscope was used.

## RESULTS AND DISCUSSION

For the first time in Romania, species *Filenchus cylindricus* and *F. misellus* are discovered and described.

*F. cylindricus* was found in soil samples which were cultivated with maize at Fundulea and in cultivated soil with potatoes, onion, garlic, parsley, at Baneasa-Bucharest.

*F. misellus* was found in cultivated soil with potatoes, onion and garlic, from Baneasa-Bucharest.

In this paper we will present the description based on morphometrical characters for these two species recorded in Romania for the first time.

*Filenchus cylindricus* (Thorne and Malek, 1968) Niblack and Bernard, 1985 (Fig. 1 A-F).

Measurements, female: Population 1 (n = 2); L = 0.62 – 0.71; a = 34 – 35.8; b = 5.9 – 6.5; c = 4.0 – 4.7; c'= 10.3 – 14.3; V = 60 – 61.6; V' = 87 – 89; VA/T = 44 – 77.4; G = 168 – 184; MB = 54; stylet = 13 – 14.5.



Population 2 (*n* = 4) ; *L* = 0. 627 ± 0. 2 ( 0.600 – 0. 650 ) ; *a* = 33 ± 5 (28.1 – 39. 4); *b* = 5.6 ± 0.9. (4.6 – 6.5) ; *c* = 4.5 ± 0. 3 (4 – 4.7) ; *c'*= 12.5 ± 2.3 (10 – 15.6); V = 57.5 ± 2.7 (53 – 60); V' = 75 ± 5.7 (68. 2 – 82) ;. *VA/T* = 63 ± 17 (44 – 79) ; G = 142 ± 32 (95 – 17) ; *MB* = 45 ± 0.8 (44 – 46); stylet = 13.2 ± 1.3 (12 – 14.5).

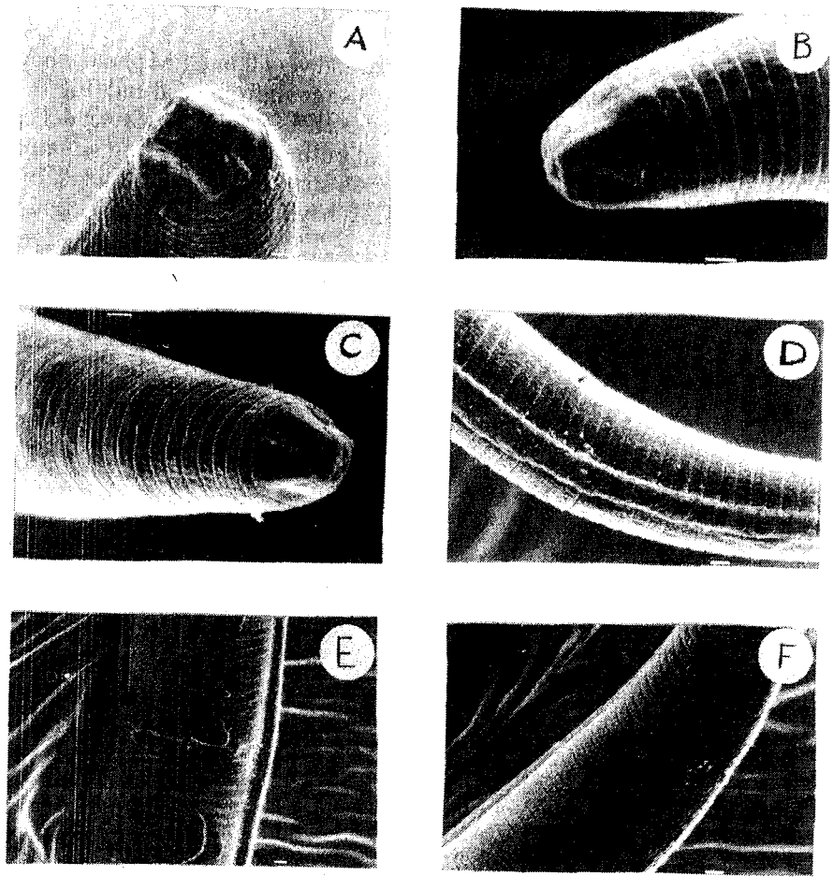


Fig. 1. – *Filenchus cylindricus*. Female A-B.C. Anterior end with beginning of lateral field. D. Lateral field. E. Vulva. F. Anus. (Bar = 1 μm for A-F)

Description: Cylindroid body, 16 – 18 μm wide. Cuticular annuli 1 – 1.1 μm wide near mid-body. Lateral field starts at 15th annulus from the anterior end (Fig.1, C). Scanning photographs show amphideal aperture as longitudinal slit beginning behind the labial plate (Fig. 1, B), followed by two small annuli, the rest of the head being smooth.

First population comes from cultivated soil with maize (Fundulea) and second population from cultivated soil with potatoes, onion, garlic, parsley (Baneasa-Bucharest). These two populations are considered as *F. cylindricus* due to the stylet length. They

are however smaller than hitherto reported (7). Also SEM has shown a smooth head except for two annuli at the top, contrary to the annulated head in Raski and Geraert (7).

*Filenchus misellus* (Andrassy, 1958) Geraert and Raski,l987 (Fig. 2, A-F).

Measurements: Females ($n = 4$); $L = 0.380 \pm 0.039(0.33 - 0.42)$; $a = 30.4 \pm 6.7$ (21,1 – 36.2); $b = 4.6 \pm 0.8$ (3.3 – 5.2); $c = 5 \pm 1.4(3.1 - 6.2)$; $c' = 8.8 \pm 3.5$ (5.4 – 13); $V = 74 \pm 6.1$ (67 – 82); $VA/T = 65.5 \pm 31$ (40 – 99) ; G = 125 ± 25 (92 – 154); $MB$ – 41 ± 3.3 (37 – 45) ; stylet = 7 ± 0.5 ( 6.5 – 7.5).

Description: Cuticle very finely annulated and very thin. SEM photos showed the lateral field with four lateral lines, starting 38 annuli from head end (Fig. 2, C). The end on view is quadrangular with rounded corners (Fig. 2, B). Stylet delicate, the knoles small but easy to observe; median bulb oval, nerve ring situated at 52.5 ± 5 μm from anterior end. Vulva transverse slight, vagina thin-walled, ovary short. Tail 64 ± 3 μm long, in the posterior half subcylindrically pointed (Fig. 2, F).

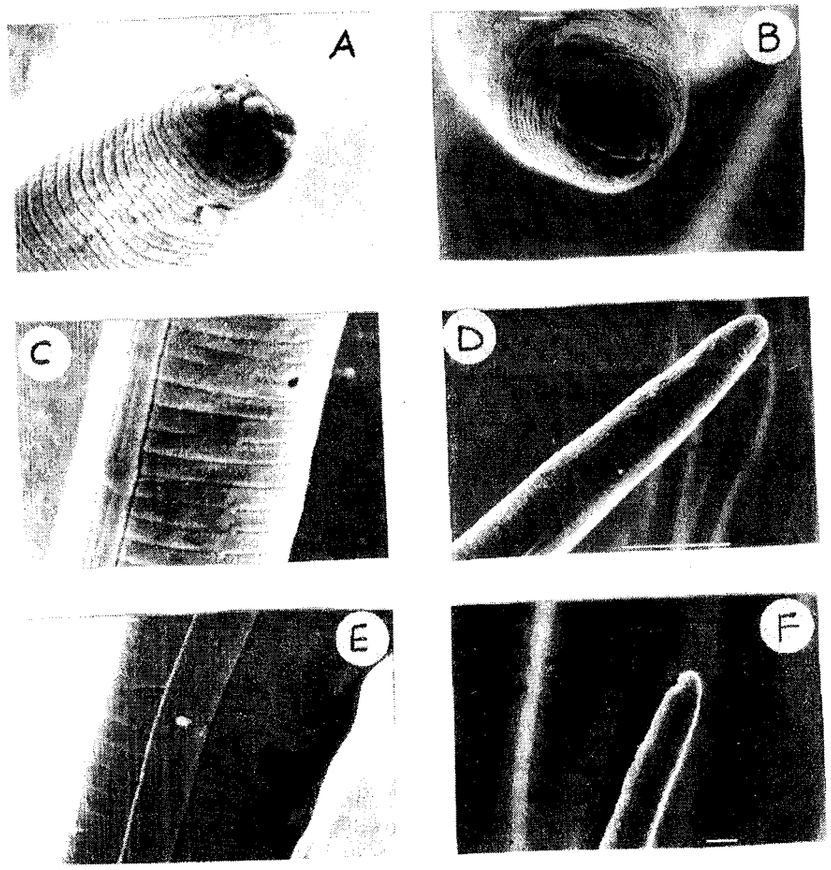


Fig. 2. – *Filenchus misellus*. Female. A. Lateral view of the head B. Face view. C. Anterior end and beginning of lateral field. D. Region of excretory pore showing deirid. E. Vulva region with plasmid. F. Tail. (Bar= 1 μm for A, B, D, E, F, 10 μm for C)



Population comes from cultivated soil with potatoes, onion, garlic and cabbage (Baneasa-Bucharest). There were noticed two small differences with Andrassy's original description; the first that the tail is shorter and the second that the vulva is more anterior [in Andrassy, V = 68.2; in Baneasa-Bucharest, V = 74 ± 6.1 (67 – 82)].

## CONCLUSIONS

*Filenchus cylindricus* and *F. misellus* are for the first time recorded in Romania.

*Filenchus cylindricus* in Romanian populations presents all morphometrical characters, the same as in literature. The body length is smaller, but this is interpreted as a variation within the specimens.

The *F. misellus* Romanian population has two small differences, as in literature: the tail is shorter and vulva is more posterior. The differences are considered with the range of variability of this species.

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# ASPECTS CONCERNING THE STRUCTURE AND THE ULTRASTRUCTURE OF THE ALIMENTARY TRACT OF SOME *BLATTA* SPECIES

GETA RÎŞNOVEANU[1], DOINA CODREANU-BĂLCESCU[2], MARIA SUCIU

The paper presents the results of a study on the morphology and on the histological and cytological differentiation of the main regions in the alimentary tract of *Blatta orientalis* L. and *Blattella germanica* L.
In the case of the two species taken into study, the gut is differentiated into several, more or less distinct segments, by variations of the diameter, by differences in the structure of the epithelium and the chitinous membrane and also by the muscular layer conformation.
The histological and cytological characterization of the morphologically differentiated regions is accompanied by nineteen original pictures.

*Key words*: alimentary tract, structure, ultrastructure, *Blatta orientalis* L., *Blattella germanica* L.

## 1. INTRODUCTION.

The present paper consists in the results of a study on the morphology and on the histological and cytological differentiation of the main regions in the alimentary tract of *Blatta orientalis* L. and *Blattella germanica* L.

The importance of these insects as discomfort producing factors and as vectors for numerous pathogenic agents - an aspect revealed by the special literature and especially the WHO publications (1) - has initially determined us to tackle the studying of these species, in order to evince some symbiont and parasites which may represent sources of infection for man. Since many of these symbiont and parasites evolve most of their life cycle in the intestinal epithelium, we have considered an approach to the structures and ultrastructure at this level to be a necessary stage, both in indemnant situations and in cases when various pathogens occur, the more so as the problem is usually neglected in special literature.

## 2. METHODS

The usual histological techniques of fixation in formalin 10% phosphate buffer and in alcoholic Bouin were used, the staining applied being haemalaun-eosine or erythrosine, alcyan blue and ferric hematoxylin Heidenhain-erythrosine-orange G. The thickness of the sections was 5-6 μ and they have been done at the level of the three regions of the intestine: the foregut, the midgut and the hindgut.

For the electron microscopy we used the double fixation procedure (pre-fixation with glutaraldehyde 2.5% and post-fixation with $OsO_4$ solution 2% in 0.1 M

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cacodylate buffer). The ultrathin sections, laid on grids, were stained with uranyl acetate and contrasted with lead citrate, according to the Reynolds method.

## 3. RESULTS AND COMMENTS

The histological and cytological differentiation of the main regions in the alimentary tract depends on their origin and predominant function.

### 3.1. THE FOREGUT (STOMODEUM)

For the two species taken into study, the foregut is differentiated into several, more or less distinct segments, by variations of the diameter, by differences in the structure of the epithelium and the chitinous membrane, and also by the muscular layer conformation. It includes the pharynx (anatomically little differentiated), the esophagus, the crop, the gizzard (the proventricle) and the cardiac valvulae (Fig. 1).

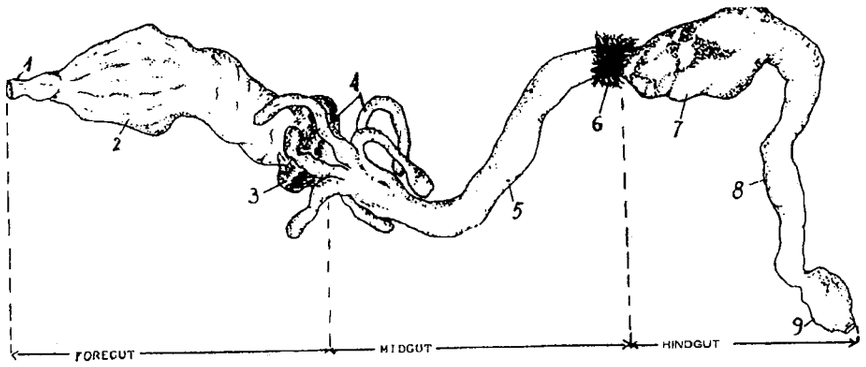



Fig. 1. – Digestive tract – Schematic diagram: 1-esophagus, 2-crop, 3-gizzard, 4-ceca, 5-midgut (tubular part), 6-Malpighian tubules, 7-dilated portion of hindgut, 8-colon, 9-rectum.

The esophagus is a narrow duct, the diameter of which gradually increases up to the dilatation of the stomodeum, i.e. the crop, its posterior limit remaining rather indistinguishable. The epithelium is unistratified, flattened and the cellular limit is frequently indistinct. The cellular cytoplasm is dense, rich in elongated mitochondria, with a spherical nucleus situated in a central position. The innermost stratum (intima) of the esophagus is thin, poor in chitin and has slender ornamentations.

The crop has the appearance of a large dilatation in the posterior part of the esophagus. When not distended by its content it is longitudinally folded and transversally wrinkled. This characteristic facilitates a gradual dilatation.

The main function of the crop is one of alimentary storage, but the analysis of its content, performed upon many insects, has revealed the existence of digestive enzymes and there is no doubt that the crop is also a place for some



digestive phenomena too (3,4). The absorbant role of the foregut is one of limited importance.

The epithelium of the crop is unistratified and flattened. The chitinous intima is usually thick and on its inner side it has strong, acicular thorns, which are better formed in the case of *B. orientalis*.

The epithelial cells have polyhedral aspect and their apical pole presents numerous invaginations of the plasmalemma. Characteristic for this zone are also the wide spaces among the epithelial cells, suggesting the presence of an extra-cellular matrix that has a role of keeping the cells together and of communication among them (Fig. 2). The cytoplasm of these cells has a granular aspect and is abundant in mitochondria and ribosomes. The nuclei of an irregular, atypical shape are big and they present chromatic agglomerations and notches. The latter are probably linked to some stages in the activity of the nuclei in question (Fig.3).

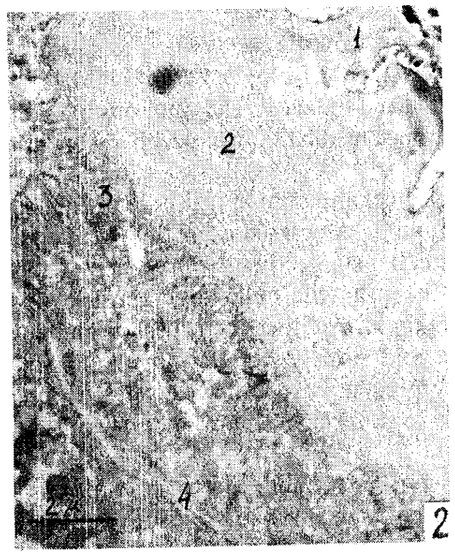
The epithelium of the crop (and of the other stomodeal regions) presents a thin basal membrane, on the outside of which there are an internal longitudinal muscular layer and a continuous external circular muscular layer. The longitudinal muscularity is disposed in groups which tend to a localization in the sinuosities of the gut wall. Slender tracheal branches penetrate along the epithelium (Fig. 3).

The proventricle is characterized by a marked development of the parietal folds, which are armored by plates, thorns and chitinous denticles differentiated in the intima (Fig. 4), and at the same time by the thickness of the external circular muscular layer that is preponderant and continuous (Fig. 5). The folds of the epithelium are oblong and very well-marked especially when compared with the ones of the crop. The *Blatta* species have six principal longitudinal folds and a variable number of intermediary folds. The longitudinal muscularity occupies the base of the big folds. In the concavity of these folds we have identified short transversal muscular fibres (Fig. 6). The gizzard seems to be an organ both for triturating and for sorting (3,4).

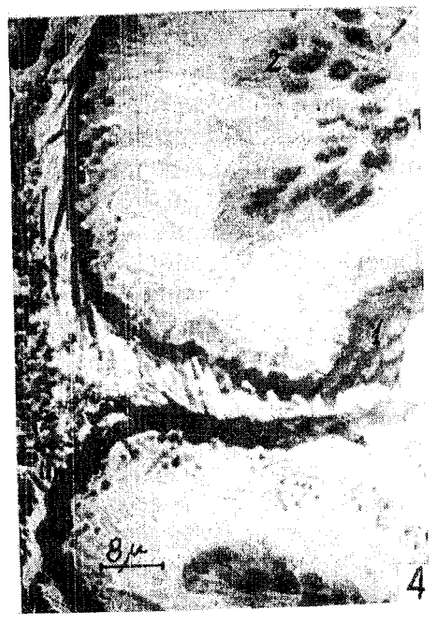
The stomodeal (cardiac) valvula is a circular twofold plait at the posterior extremity of the foregut, and it appears as an invagination in the interior of the mesenteron (Fig. 6). In the main, it prevents the return of the aliments from the mesenteron back into the anterior intestine.

### 3.2. THE MIDGUT (MESENTERON)

The midgut is one of endodermic origin and in some works it is also called the chyliferous ventricle or stomach (2,5). Its anterior limit is represented by the base of the external fold of cardiac valvulae a zone where the stomodeal intima ends. As for the posterior limit, it can be situated not far from the opening of the Malpighi tubes. For the species taken into study the mesenteron consists of six caeca and a tubular part (Fig. 1).







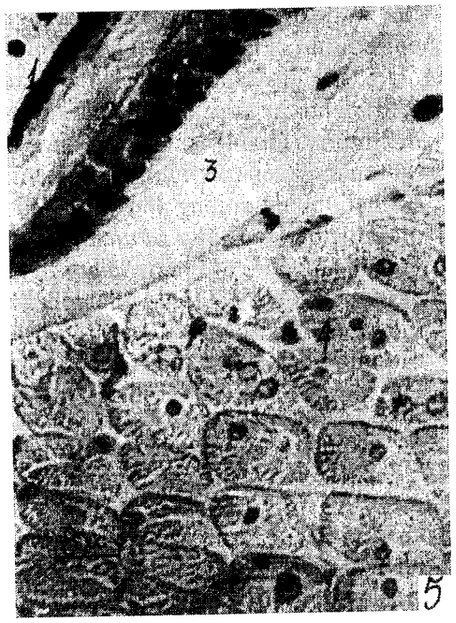



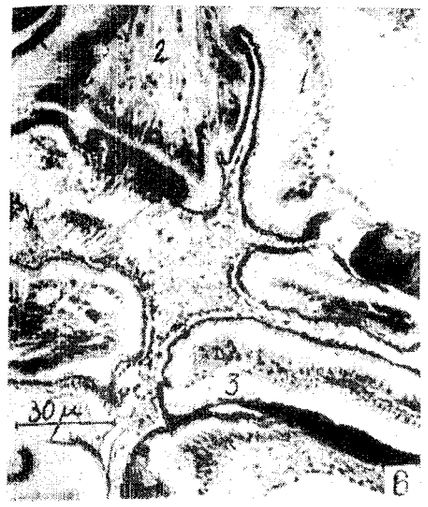

PLATE I

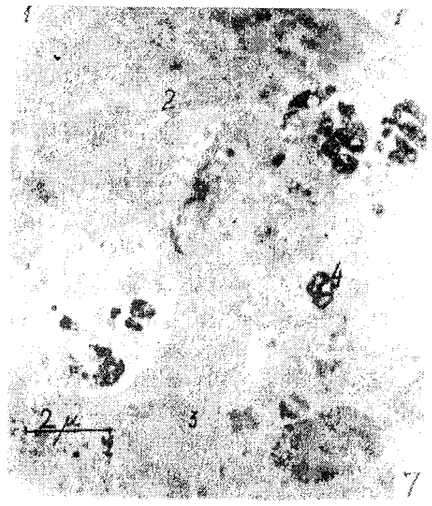
Figs. 2-3. – Crop wall (electron microscopy): Fig. 2 – *B. orientalis*: 1-acicular thorus, 2-chitinous cuticula, 3-plasmalemmal invaginations, 4-intercelular space; Fig. 3 – *B. germanica*: 1-longitudinal muscular layer, 2-tracheal trunk, 3-epithelial cells, 4-mitochondria, 5-nuclei.

Figs. 4-5 – Gizzard (histological sagittal sections) - *B. orientalis*: Fig. 4: 1-plates, thorns and chitinous denticles in intima, 2-epithelium;

Fig. 5: 1-chitinous cuticula, 2-epithelium, 3-longitudinal musculature, 4-circular musculature.







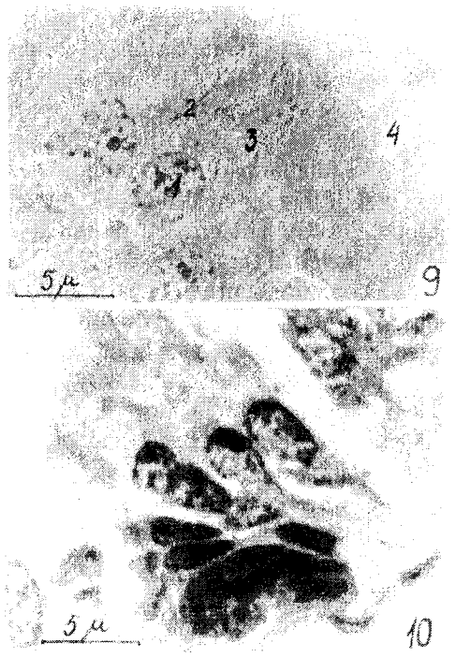



PLATE II

Fig. 6 – Stomodeal valvule (histological sagittal section) - *B. orientalis*: 1-epithelium, 2-transversal muscular fibers, 3-the twofolds of cardiac valvule.

Fig. 7 – Caeca cells (ultrastructure) - *B. germanica*: 1- microvilli, 2-rugous endoplasmic reticulum, 3-mitochondria, 4-phagosomes.

Figs. 8-10 – Midgut (histological cross sections) - *B. orientalis*: Fig. 8: 1-muscular layer, 2-villosities, 3-regeneration crypt, 4-peritrophic membrane; Fig.9: Columnar cells: 1-nucleus 2-cell plasmalemma, 3-secretion vacuole, 4-microvilli; Fig. 10: Crypt.

On the interior side of the caeca there are longitudinal folds, formed by the joining of two epithelial layers, the basal laminae of which are thus very close to each other, for the connective tissue is poorly represented. At the base of the fold the two basal laminae are farther from each other, and therefore the section acquires a triangular shape. Thick tracheal trunks penetrate the connective tissue at this level acting as a skeleton that supports this structure. The longitudinal folds are very tall; they enlarge the surface and at the same time they considerably narrow the interior of the ceca.

The epithelial cells of the caeca are very elongated and their height is variable. In their compact and granular cytoplasm there are numerous mitochondria and a dense network of rugous endoplasmic reticulum, which is even more abundant in the apical zone of the cells, a zone where the nuclei are also situated. At their apical poles, the cells present microvilli. We also mention the presence of some infrastructures which can be either lysosomes or phagosomes; they are big and they appear to be one, two or even three per cell (Fig. 7 ). The basal membrane of the epithelial cells is hardly distinguishable. As the special literature on the topic is unanimous in ascertaining the opinion that this is a zone where only pinocytosis phenomena - and not inclusions of solid particles - take place (2,4), the occurrence of these structures might be associated to the processes of cellular degeneration, followed by an intense regeneration of the epithelial zones in question on account of the cells in the regeneration crypts at this level.

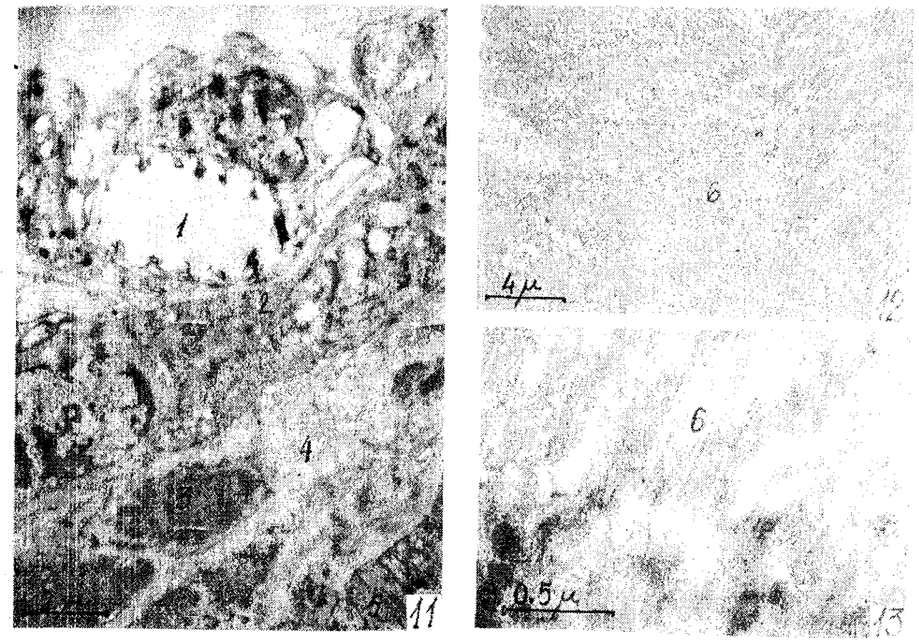
The tubular part of the midgut is constant in diameter all along (Fig. 1). At the level of this segment there are numerous villosities separated by regeneration crypts (Fig. 8). The circular muscular fibres are internal while the longitudinal ones are external (Fig. 11).

The epithelium is considerably thicker than the one of the foregut and it consists of tall, frequently cylindrical cells, with big nuclei usually situated in their apical zone. The cytoplasm of these cells has various aspects depending on the age of the cell and also on the intensity of the cellular activity. Thus, young cells have a dense granular, abundant in ribosomes and ergastoplasmic lamellae cytoplasm, while in the case of mature cells it acquires a vacuolar aspect, the vesicular dilatations of the endoplasmic reticulum being predominant. For this intestinal segment the cellular limits are clearly distinct.

The main elements of the epithelium are columnar, tall and thin cells (Fig. 9). They are placed right on the basal lamina, which, taking into consideration its fibrillar structure, seems to be a non-cellular tissue of connective nature, not differing from the ones of the foregut and hindgut. At the basal region of the epithelial cells the plasmatic membrane sends numerous invaginations into the cytoplasm, thus considerably increasing its surface (Fig. 11). All columnar cells have on their apical surface cylindrical microvilli (Fig. 12), run through by bundles of longitudinal fibrils which may also penetrate the underlying cytoplasm (Fig. 13).

The cytoplasm of the columnar cells contents microtubules and numerous mitochondria disorderly dispersed in the middle and apical zone of the cells, while in the basal region the mitochondria are elongated they are displayed in rows perpendicular to the basal membrane and they are separated by plasmalemmas.





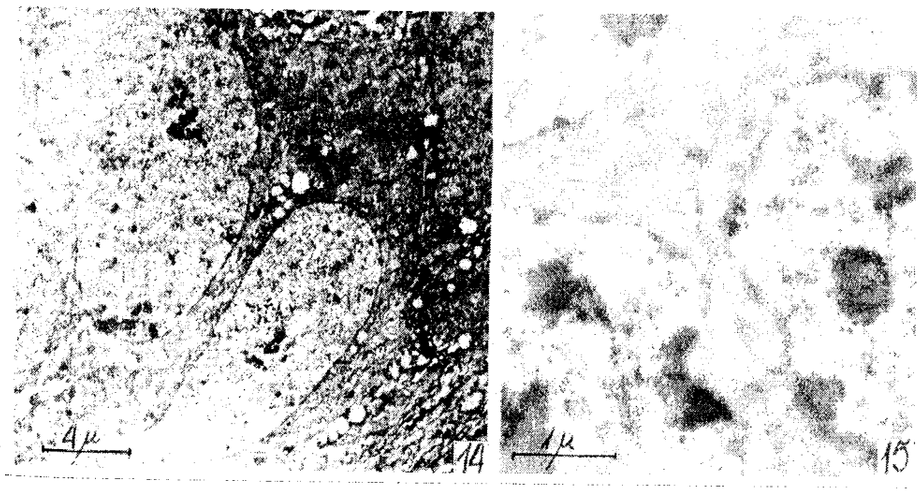




PLATE III

Figs. 11-13. – Midgut wall (ultrastructure) - *B. germanica*: 1-tracheal trunk, 2- longitudinal muscular layer, 3-circular musculature, 4-connective tissue, 5-columnar cell; 6-microvilli details.
Figs. 14, 15. – Epithelial cells (ultrastructural details): Fig. 14 – *B. orientalis* crypt cells; Fig. 15 – *B. germanica*: columnar cells.



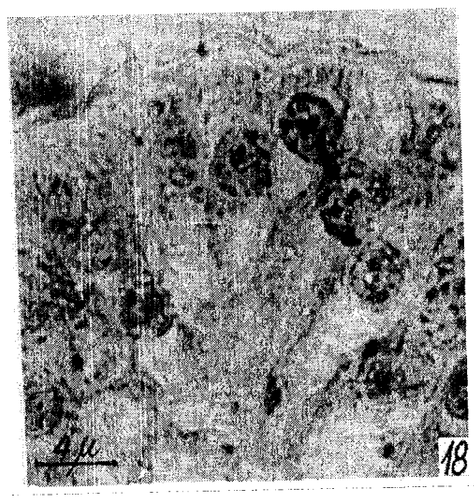




PLATE IV

Figs. 16,17 – Hindgut (sagittal histological sections) *B. orientalis*:
Fig. 16 – Piloric valvule: 1-circular musculature, 2-epithelium; Fig.17 – Dilated portion:musculo-connective sheath, 2-epithelium, 3-microvilli.
Figs. 18,19. – Hindgut-The colon epithelial cells (cross sections): Fig. 18 – *B. orientalis*-histological aspect; Fig. 19 – *B. germanica*, ultramicroscopic details.



Frequently they agglomerate along the intercellular limits. There are also the ergastoplasm and the vesicular cisterns of the endoplasmic reticulum. The cells often contain granules, and vacuoles of secretion of variable dimensions, but they seldom contain lysosomes. The nuclei are big and situated in the center or towards the apical part of the cells (Fig.9); they have an irregular contour in tangential view (Fig. 15). Sometimes they contain crystalloid structures, apparently of proteic nature, the presence of which may suggest a metabolic activity of the epithelium (3).

The columnar cells originate from the regeneration crypts regularly spaced out in the epithelium of the midgut (Figs. 8,10). They are made up of "nests", or agglomerations of small cells, with homogeneous cytoplasm and pyriform nuclei, whose cellular limits are often almost indistinct (Fig. 14). A crypt usually contains a single initial cell that preserves the capacity to divide by mitoses and to produce the other cells of the crypt which are incapable of multiplication. They contribute to the regeneration of the epithelium. The columnar cells are the ones to take part in the digestive phenomena (secretion and absorption). The small basal cells in the crypts are the elements of replacement that gradually substitute those elements, worn out as a result of their secretory activity and those removed during shed.

The epithelium of the midgut is in a permanent more or less intense process of disintegration, accompanied by the renewal of the destroyed elements. Just before the sheds the process intensifies.

Some authors (3,5) have also depicted for the mesenteron of certain species of insects a type of the so-called "calyx-shaped" cells that are not to be found, however at all the species. These cells are not at all rich in ergastoplasm, but they present a very abundant variety of cytoplasmic extensions. As these cells have not been caught in our sections on their mediosagittal plane, but only, possibly on the tangential one, we cannot positively state the presence of this cellular type in the epithelium of the mesenteron for the *Blatta* species taken into study.

The contents of the midgut is isolated from the epithelium of the stomach by a thin cover membrane, which is called "the peritrophic membrane" (2,3,4) (Fig. 8). In the case of the *Blatta* species it has a tubular shape, a multilamellar wall, it is thin and transparent.

### 3.3. THE HINDGUT (PROCTODEUM)

The last segment of the alimentary tract – the hindgut – has an ectodermic origin. The information regarding the structure of the hindgut for diverse groups of insects is still heterogeneous and incoherent, the various regions not being unanimously recognized. In the case of the *Blatta* species, the proctodeum is separated from the mesenteron by a proctodeal or pyloric valvula. The Malpighi tubes open right above this valvula; (Fig.1).

For the *Blatta* species the posterior intestine can be further subdivided into five successive regions, namely: the prevalvular segment which is short providing the junction with the midgut; the pyloric valvulae; a very dilated portion; the colon; the rectum. Each may vary in size from one genus to another.

The prevalvular segment is short and it has a diameter similar to the one of the mesenteron. The epithelial cells at this level are tall, cylindrical the nuclei being centrally situated. There are vacuoles in the cytoplasm and the plasmalemma at the apical pole is evaginated, forming microvilli. A thin cuticula lies over them.

The pyloric valvula is an epithelial plait and in the interior it presents folds armored by chitinous cuticular plates (Fig. 16). A circular muscle (pyloric sphincter) takes part in the occlusive processes and a longitudinal muscular layer has an antagonistic role. At the exterior the pyloric valvulae cannot be distinguished from the rest of the hindgut.

The proctodeum considerably dilates right beneath the pyloric valvulae (Fig. 1). At the level of this dilatation there are some folds, the presence of which is connected with the gradual modifications in volume. The epithelium of this region is thick and unistratified, with very slender cylindrical cells. Their height is variable, the aspect of the epithelium at this level therefore being a papillary one. The nuclei are usually situated in the basal half of the cells and it is only in the central zone of the papillae that they are situated centrally or towards the apical pole (Fig. 17). At the apical pole the cells present long microvilli and the intima is thin simple and not at all rich in chitin (Fig. 17).

The colon represents about half the length of the proctodeum. At this level the unistratified epithelium consists of cylindrical cells, considerably taller than the ones in the foregut (Fig. 18).

By electron microscopy it has been revealed that the proctodeal cells have a structure that does not substantially differ from the one of the columnar cells in the mesenteron. The proctodeal cells belong to the absorptive type, are clearly contoured and they have a bordering of subcuticular microvilli. There are also basilar cytomembranes and mitochondria oriented towards the gearing with the vicinal cells.

The bordering of the cells may appear visibly striated, the intima is thin, supple and unlike the one of the stomodeum it is always permeable to water (Fig. 19). The nuclei usually multiply by amitosis. The cytoplasm is run through by epitheliofibrils and there are frequent intracellular tracheal terminations.

Some studies (3,4) mention the presence at the level of the proctodeum of some "endocrine" cells, producing a proctodeal hormone.

Cells of similar characteristics have been identified by us at the level of the proctodeum for the two species taken into study, these cells being more numerous in the case of *Blatta orientalis*. We cannot supply a definite answer regarding the nature and function of the cells in question. In the interior of the hindgut there are frequent agglomerations of bacteria many of them being probably symbionts.

The muscular fibers of the proctodeal muscular stratum have an often irregular distribution and in certain zones they may be missing.

Roughly, we can distinguish a layer of external longitudinal fibers and a layer of internal circular fibers, but there are also frequent situations when the longitudinal fibers appear inserted amid two layers of circular fibers. The longitudinal fibers are usually grouped into six bands. In the anal region the circular fibers form a sphincter. In the posterior zone, the muscular layer is accompanied by extrinsic



muscles originating in the abdominal wall; they are the suspensory and distending muscles of the hindgut.

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# HEPATOMA AND OKER ASCITE PRESENCE. AN ULTRASTRUCTURAL STUDY

D. MIŞCALENCU, FLORICA MAILAT, CAMELIA IANCU

The presence of solid tumour – $RS_1$ – hepatoma – and ascitic tumour Oker, induces in bone marrow an increased number of mast cells and eosinophils which exhibit some modifications: the mast cells reveal an intensive degranulation and the specific eosinophils' granules are altered.

## INTRODUCTION

The tumour-bearing animals exhibit a number of qualitative and quantitative modifications of some cellular lines in hematopoietic tissue.

First of all a remarkable and violent reaction of mast cells and eosinophils comparative with other cells was observed.

The $RS_1$ hepatoma is an uncapsulated tumour, not metastases but fast increases and it is strongly vascularized; so the necrosis areas appear quite late. Oker ascite has a fast evolution leading to animals' death. By their presence and their toxic products the tumours induce a strong reaction of immunitary system cells.

The basophils and mast cells are actively involved in the pathological process induced by tumours. They exhibit some morphological and biochemical modifications in the presence of non-self products; in consequence they release mediators and they degranulate (1). They are in abundance in delayed and transitory hypersensitivity reactions and also proliferate in the presence of bacterian products and of foreign proteins (2).

The eosinophils as effector cells play an important role in the IgE-depended hypersensivity, they release cytotoxic proteins (2) or mediators. They stimulate or inhibit other hematopoietic cells by some cytokines; the eosinophils release, for example, TFG – alpha and TGF – beta as well.

The number of neutrophils is increased in the tumour presence; their activation, via cytokines and/or integrins (4), leads to their migration and biochemical transformation.

The aim of our study was to establish the ultrastructural modifications of some cellular types from bone marrow in the presence of two kinds of tumours: $RS_1$ hepatoma and Oker ascite.

## MATERIAL AND METHOD

The white Wistar rats were subcutaneously inoculated with $RS_1$ – hepatoma tumoural macerate and others were i. p. inoculated with ascitic liquid.

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The bone marrow smears were stained by May-Grünwald-Giemsa method and were examined on optical microscope.
The bone marrow fragments were fixed in glutaraldehyde in buffer cacodylate 0.1 M for 2 1/2 hours, washed in the same buffer, postfixed in $OsO_4$, embedded in Epon, sectioned at 40 millimicrons and double stained with uranyl-acetate and lead-citrate. The sections were examined on the Philips electronic microscope.

## RESULTS

The first observation was the increased number of mast cells and eosinophils: it was almost by six times greater than the number in control animals. The mast cells exhibit an advanced degranulation state, concomitant with granule formation inside blastic cells (Fig. 1).

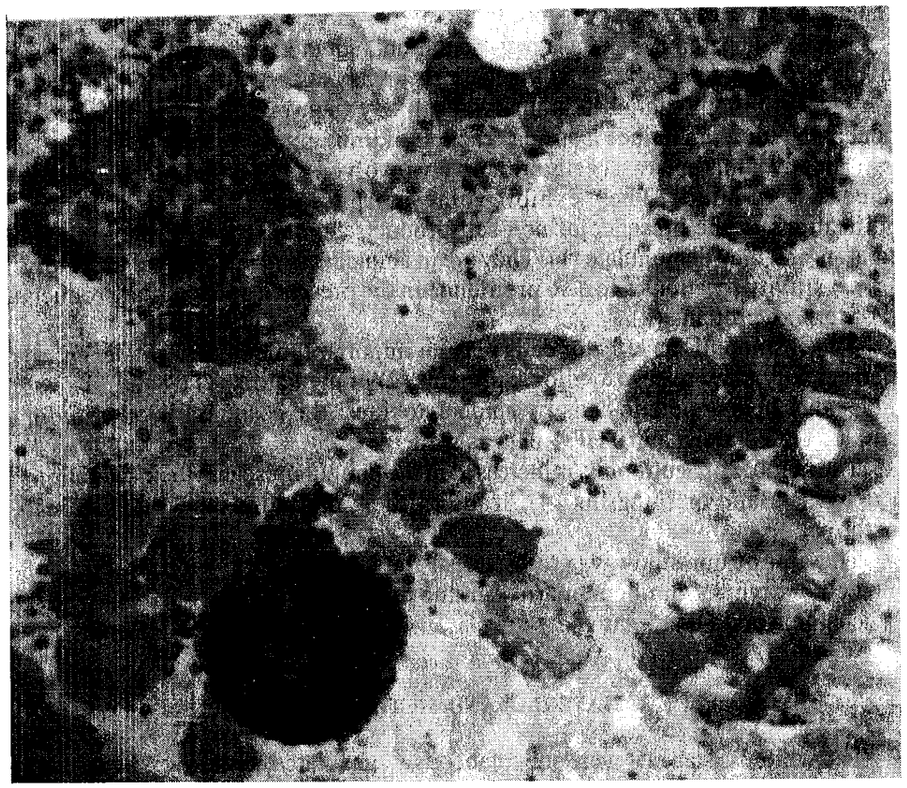

Fig. 1. – The mast cells in bone marrow of the $RS_1$ hepatoma-bearing rat; The cells exhibit a clear process of degranulation.



The ultrastructural analysis of bone marrow reveals an active process of elaboration of these cellular types.

The samples examined reveal the eosinophils in all their evolution stages of maturation and their characteristic granules exhibit some structural alterations. In the incipient stadium of granules' formation, the crystalloid was electronodensely surrounded by an electronoclear area; others, also incipient granules, have not organized the central crystalloid; at last, the mature granules are surrounded by an electronoclear halo (Fig. 2).

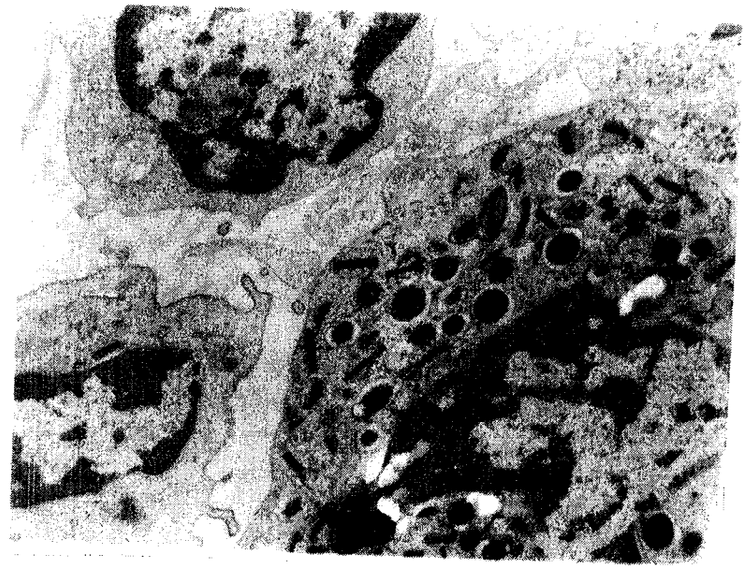

Fig. 2. – An eosinophil in bone marrow of the ascite Oker-bearing rat; the mature granules exhibit ultrastructural defects which reveal the absence of the characteristic material which should surround normally the Charcot-Leyden crystalloid. The immature granules have an electronoclear halo around.

Regarding the nuclear chromatin aspect, the eosinophils appear as mature ones (Fig. 2), but their granules seem to be in an incipient stadium of differentiation (Fig. 2); the cytoplasm reveals mitochondria with a few crists and numerous ribosomes associated or not with ER cisternae.

The plasmacytes exhibit a hypertrophiated RER with dilated areas inside containing a material with medium electronodensity; their presence is the result of reaction to tumoural toxins (Fig. 3).

The neutrophils are distinguished by the abundance of their blastic forms but they do not exhibit significant ultrastructural modifications.

The tumours' presence suppresses hematopoiesis but it can induce the activation of the cells implied in removing of toxic effects, as well. This presumes an active protein synthesis with the release of cytotoxic products and /or cytokines.

Fig. 3. – A plasmacyte in bone marrow of the $RS_1$ hepatoma-bearing rat. There are observed the RER cisternae with dilatations containing a medium electronodense material.

## DISCUSSION

The correlation of results regarding the numerical evolution and ultrastructural modifications of some bone marrow cells suggests that the tumours' presence induces an active production of the cells implied in the immunitary system. This requires an increased production of these cells; this increased production is a consequence of their activation as a rejoinder to the tumoural toxic products.

The basophils and mast cells are probably activated via IgE and their degranulation process is dependent on $Ca^{2+}$ influx (1.6). The increased number of mast cells can be explained by a mechanism which depends on I1-3 as mediator (5). It is known that in some tumours, for example neurofibroma, the c-kit gene is stronger expressed and associated with an increased number of mast cells (5). The CD4 lymphocytes also stimulate secondary the mast cell growth (7) via I1-9. Finally, the mast cell presence in tissue, as a barrier, facilitates the affected situs (2).

The eosinophils have directly cytotoxic activity and are also a cytokine source (TGF-alpha and TGF-betha); they are involved in the tumoural toxins neutralization (3). Their numerical increase and the interconnexions with mesenchimal and epithelial cells can intervene in tumour vascularization and, this provide them the viability (8).



The neutrophils are also required in these defense processes, they are activated either by TNF released by macrophages (9), or by their own migration signals (4); the neutrophils' integrins are receptors for some endotoxins, they seem to start the signals for migration and, they may be the receptors of tumoural toxic products (4).

The lymphocytes and macrophages are activated as well, and they intervene in those defense molecular events by their cytokines' production – specially I1-1, I1-6 – or by phagocytosis (10, 11).

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# VIRUS-LIKE PARTICLES IN THE PARENCHYMA LIVER CELLS OF CYCLOPHOSPHAMIDE TREATED RATS

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Cyclophosphamide induces disintegration of intracytoplasmatical membranes which have as effect the almost whole disappearance of ER, Golgi complex and also pronounced modifications of hepatocytes' mitochondria. If in some cells the nucleoplasmatic rate is relatively normal, in others, this is seriously affected to the nucleus detriment, nucleus which has at its periphery a halo with mitochondria inside. In the nuclei there can be seen virus-like particles which appear in the peripherical nuclear chromatin; the nucleus has not a membrane, so the particles are released in the cytoplasm. The particles have not an electronodense core and their membrane bears granules.

## INTRODUCTION

No other virus in natural infection produces such a quantity of envelopes, as HBV (hepadna B virus) does. This virus has an integrated DNA genome.

The viral spherical particles (20 nm) contain almost exclusively the gene S codificated polypeptides so, in primary infection, the infected patients manifest the anti-pre$S_1$, anti-pre$S_2$ and anti-pre$S_3$ reactions (18).

The hepatocytes of active cirrhosis patients contain many copies of double stranded-DNA-HBV in their nuclei, and on their surface, high levels of $HB_eAg$ (8). The higher titres of pre-$S_1$ and pre-$S_2$ in the patients' blood are well correlated with the X protein appearance, which is considered to be a transactivator of HBV genome.

The cellular line of human hepatoma huGK-14 produces surface Ag. particles HBV. The spherical particles (23 nm) are $HB_sAg$. particles which remind the $HB_sAg$ particles in the blood of HBV-bearing patients (13).

In the liver of two chronic active hepatitis patients immunodepressed, there were discovered virus-like particles (20 nm) a half of hepatocytes' nuclei contained viral particles (18-25 nm) without nucleoid (electronodense core) which though was exceptionally present. These empty particles were disseminated in voluminous clusters, among the chromatin aggregates, or ordely, inside nucleoplasma, like some chains.

In the hepatocytes cytoplasm, in the mesenchymal cells, or in the normal liver of both patients, there were not detected viral particles (7).

If hepadna virus is not mentioned in the rats (2), in the mice hepatitis virus type 4 produces few quantities of IL-2 and IL-3 cytokines, in infected spleenical cells (14).

For the beginning, the cyclophosphamide was considered a tumoral cytostatic, because its action on hexaatomic ring on which rends and releases

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N-iperide, following the phosphatase and phosphomidase hydrolysis inside the cancerous tissue.

Frequently, the cyclophosphamide was used in combination with other drugs for the purpose to diminish its toxic effect (1, 13, 3, 19, 20, 9). Subsequently, it was demonstrated that the cyclophosphamide is transformed in the liver' microsomes and the resulted metabolists inhibited, by their toxicity, the cellular proliferation (10, 5).

However, in the treatment with bisulfan and cyclophosphamide of multiple myeloma (a good combination for patients with allogene bone marrow transplant), the toxic action of both drugs induces diverse diseases among the liver venes occlusion (6).

For prevention of diabetes induced in mice with cyclophosphamide, it is admitted that the drug selectively influences T cells regulation, the toxic action altering the regulation system. It supposes that because the cyclophosphamide injuries DNA and stops the CD4+ cells activity (17). By its action on cellular DNA, the drug may influence integrated DNA viral genome, too, or may activate the proto-oncogenes which on their line may initiate the transformation. In this idea, it was demonstrated that the aflatoxin which stimulates HBV genome in the liver cancerization (12) by activation of integrated HBV, may start the activation of myc proto-oncogene, too.

The cyclophosphamide induces also the survival of rabies virus in normal human cells (16), and increases also the herpes simplex virus production, in mice' CNS which induces the animals' paralysis; in exchange, the cyclosporin diminishes the disease (4).

The ultrastructural analysis of the rats' hepatocytes treated with cyclophosphamide reveals the modifications which presume the existence of a viral genome which can be activated by the drug and suggestively by the appearance of at least incomplete viral particles.

## MATERIAL AND METHOD

The adult Wistar rats received i. m. cyclophosphamide (N-phosphate N chloretyl-N-O-propylene diamide) and after 3 days they were sacrificed. Small liver fragments have been fixed for 2 hours in 2.5% glutaraldehyde in 0.1 M buffer cacodylate (pH 7.4), washed in the same buffer overnight at 4 °C, fixed in OsO4. The fragments were then embedded in epon, sectioned at 40 millimicrons, double stained with uranyl-acetate and lead-citrate. The sections were examined on Hitachi electronic microscope..

## RESULTS

Beyond the modifications induced in intracytoplasmatical membranes and plasmalemma by cyclophosphamide, the glycogen disappearance from the cells seems to be one of the visible consequences of the drug action. Other marked



modification is the almost whole disappearance of ER which is represented only by few perimitochondrial cisternae.

The modifications of mitochondria begin with minor structural alterations and finish with total lysis and some of them are totally electronodensificated.

The nuclei of hepatic parenchyma cells, in the first line of hepatocytes, also exhibit remarkable modifications; in some cells, the nucleo-plasmatic rate is normal, but the nucleolemma is affected on large distances in which the external laminae dissapears; in other areas it moves off by internal laminae and forms dilatations which may represent the prelude of its disintegration.

The chromatin is quantitatively normal and is peripherically disposed. In other phase of hepatocytes' evolution, the nucleus in spite of fact that it conserves its size, it impoverishes in chromatin. On the other hand, the hepatocytes which were strongly affected by the drug show an almost pycnotic nucleus. It is important to remark that the nucleolemma is absent and in its place there is a free space.

The chromatin of normal or pycnotic nuclei represents the place in which appear virus-like particles which are released in the cytoplasm. In the first moment, some electronuclear spheres with incomplete contour appear, maybe because of an incomplete assembly of constitutive elements, or of the fact that the section is not made in the sphere center.

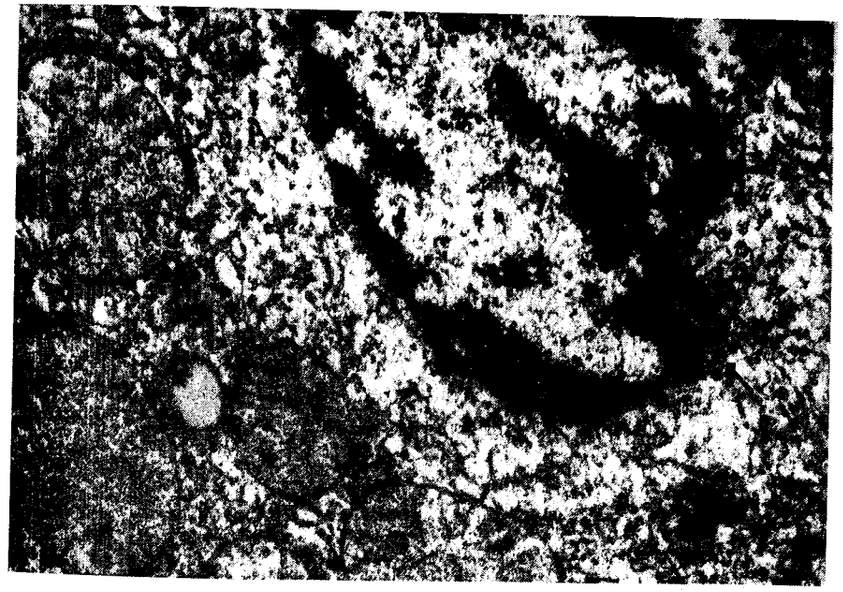

Fig. 1 – The diminished nucleus of a hepatocyte; it has around a large electronoclear space. The chromatin which is in direct contact with the cytoplasm exhibits virus-like particles in different assembling phases up to their release in the cytoplasm (arrows).

Between the sphere and the chromatin it is an electronoclear ring which separates complete or incomplete sphere, by the surrounding chromatin (Fig. 1). In the incomplete separation there are bridges between central sphere and surrounding chromatin; these spheres with granules on their surface are releasing from the chromatin electronodense material to the cytoplasm (Fig. 1,2). The granules look like some expansions of some viral types; and, on the other hand, they have not the core, the fact which reminds the hepadna B virus (20 nm) (HBAg).

Fig. 2 – One nucleus with a little chromatin quantity which is in direct contact with the cytoplasm and from which detach virus-like particles with granules on their surface (arrows).

On the other cellular types of the liver, or in the cells which transit the liver (blood cells) there are also particles like these; such a tendency of virus-like particles formation show also the nuclei of billiary pipe cells, mainly in the deep incisions of nuclei where the nuclear materials are massively discharged.

The melanocytes which appear in the rat liver following the drug administration exhibit also, in nuclear chromatin, the tendency to form virus-like particles.

## DISCUSSIONS

In spite of the fact that it is still used in anticancerous treatments, the cyclophosphamide is known as an aggressive toxic which acts by its metabolites on normal tissue too, especially on the liver (10,5).

In some studies, it was demonstrated the cyclophosphamide effect on the liver, where it induced remarkable modifications (6) manifested by an immunologic depression (17).



Because the drug acts on the cellular DNA (12) we suppose that beside the grave modifications of cells' structures, the cyclophosphamide activates directly via proto-oncogene way, the initiation of viral integrated genomes in rat hepatocytes activation. This idea is sustained also by the fact that this drug stimulates the viral replication activity (16,4).

The virus-like particles which we described in liver cells of the rats treated with cyclophosphamide are similar with viral particles from hepatocyte nuclei of chronic hepatitis B patients (HBV) (7).

The viral particles in nuclear chromatin are empty and granulous on the surface, exactly like human hepadna particles. It is important to underline that in the nuclei of normal rats and human hepatocytes, these particles are absent (7). In exchange, they appear in the human hepatoma cells huGK-14 and hepatocytes of patients with active cirrhosis; they are HBAg, particles (23 nm) formed, by the polypeptide codificated by S gene, of hepadna virus B (13, 8).

It is possible that the empty particles in rat liver cells to be an expression of HBAg type, of a possible HBV genome integrated in cellular genome of rat hepatocytes which express following the stress induced by cyclophosphamide (8, 13, 7).

On the other hand, the pycnosis of hepatocyte nuclei may be induced by continuous release of virus-like particles.

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# THE ULTRASTRUCTURE OF ENDOCRINE CELLS IN MOUSE GASTROINTESTINAL MUCOSA

ELENA POPOVICI and T. TRANDABURU

Eleven endocrine cell types (D-, $D_1$-, EC-, P-, A-like-, ECL-, X-, G-, I-, K- and S cells), included in the current international classification of gastro-entero-pancreatic (GEP) endocrine system, were identified in the mucosa of stomach (fundus, pyloric antrum) and upper small intestine of mouse. In addition, a type with distinct ultrastructural characteristics, previously described by other authors in calves abomasum, was observed in the stomach fundus of mouse.
The D-, $D_1$-, EC- and P cells were present in all studied gastrointestinal regions. The A-like-, ECL-, X cells were found only in the stomach fundus, while the localisation of G cells was restricted to the pyloric antrum. The I-, K- and S cells were identified exclusively in the upper small intestine. The ultrastructural features of the identified cell types are presented in connection with the findings of other authors. Diameter averages of granules in identified endocrine cell types ranged from 150 nm (P cell) to 500 nm (ECL cell).

## INTRODUCTION

Numerous endocrine cells are scattered in mammalian gut mucosa. [1, 3, 7, 8, 12, 17, 20, 27]. They form a diffuse multihormonal gland [8, 21, 25, 27], the most voluminous endocrine gland according to PEARSE (1977) [ 21]. Their secretory products are of peptidic nature with specific functions in the coordination of diverse activities of gastointestinal tract [8, 21, 25, 26]. Accordingly, the types of gut endocrine cells are highly variable [3, 7, 12, 20]. Thus, based on their histochemical [2, 15, 16, 25], ultrastructural [1, 3, 7, 10, 11, 24, 27], and immunochemical [5, 18] features more than 22 enteroendocrine cell types have been described in mammalian gut [26]. The most investigated species are rat [5, 6, 9, 10, 11, 14], rabbit [3, 13, 15, 16, 22], and human [2, 7, 12, 24, 27], while only a few papers have been devoted to the mouse. Only G cells [18, 19], EC cells [9, 28], ECL cells [9] were studied in the mouse stomach [14]. No quantitative study has yet been conducted on secretory granule dimensions. Taking into account the penury of data on the enteroendocrine cell types of mouse, an animal currently used in laboratory experiments, we considered useful to undertake the present investigation in an attempt to classify them.

## MATERIALS AND METHODS

Adult white mice of both sexes, raised in the Institute of Biology, were used. Fragments of stomach (fundus, pyloric antrum) and intestine (duodenum, jejunum), were processed immediately after excision as follows: fixation 2 hours at 4 °C in

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2.5% glutaraldehyde, in 0.1 M phosphate buffer (pH 7.4), postfixation in 1% $OsO_4$ prepared in the same phosphate buffer, dehydration and embedding in Durcupan ACM (Fluka, Buchs.). The ultrathin sections obtained on a Tesla BS-490 A ultratome were double stained with uranyl acetate [29] and lead citrate [23]. The sections were examined in a JEM-7 electron microscope (50 kv).

The ultrastructural features were the most useful criteria for the recognition of the cell types [1, 3, 7, 26, 27]. Another helpful criterion was the secretory granule sizes which were statistically analysed. Measurements were carried out according to LEFRANC et al. [13, 15] and OOMORI [20], by one operator, on electron micrographs of the same magnification (×22.100) by selecting the greatest diameter of each granule. The results were given as mean ±SEM, statistical comparisons of the data were performed by using the Student's test. The differences between mean values, corresponding to $p < 0.01$, were considered significant. Average values of measured diameters of granules in one cell were corrected by the following formula proposed by Buffa et al. [1].

$$D = \frac{4}{\pi} \times d$$

$d$ = mean of measured granule diameters

$D$ = corrected mean of granule diameters

## RESULTS

The endocrine cells of mouse alimentary tract appear single or gathered in small clusters (2-4 cells). In stomach, they are distributed at the base or in the intermediary portion of gastric glands among parietal and chief cells. Similarly, they occur in the intestine, in the deeper layers of Brünner and Lieberkühn glands, among enterocytes.

Twelve types of gastrointestinal endocrine cells were identified according to the secretory granule aspect and size (Table 1). D-, $D_1$-, EC- and P cells (Fig. 1; A, B, C, D) were found in all studied gut areas. Among these types EC cells were the most frequently encountered, while the incidence of $D_1$- and P cells was very low. D cells were more numerous in the stomach than in the intestine. In stomach A-like-, ECL- and X cells (Fig. 1; E, F, Fig. 2E) were exclusively detected in the fundic region, whereas G cells (Fig. 2A) only in the pyloric antrum. In stomach fundus an endocrine cell type with distinct granule morphology (Fig. 2F), but still not included in international classification of GEP endocrine system, was identified. I, K and S cells (Fig. 2; B, C, D) were localised exclusively in the intestine.

Granulometry. The histograms of granule diameter frequencies for each identified cell type are shown in Fig. 3. Granule diameter averages varied from type to type cell, ranging from 150 nm (P cell) to 500 nm (ECL cell) (Table 2). The increasing variation of these is illustrated in Fig. 4 and Table 2. The smallest average value (P type) and the greatest ones (ECL- and EC types) were significantly different against means of all other cell types (Table 2, Fig. 3; A, B). The statistical significance of difference was more useful in the case of D- and $D_1$ cells (Fig. 3C) which displayed secretory granules with similar morphological features (Fig. 1; A, B).



*Table 1*

The distribution of endocrine cell types and morphology of their secretory granules, in mouse gastrointestinal mucosa

| Fig | Type | Cell distribution | | | | Granule characteristics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stomach | | Intestine | | Shape | Osmiophily | Content | Halo | Measured diameter (nm) | Calculated diameter (nm) |
| | | F | PA | D | J | | | aspect | | (mean±SEM)(N) | |
| 1–A | D | + | + | ± | ± | round | slight | homogenous | A | 209±3.82 (235) | 266 |
| 1–B | $D_1$ | + | + | + | + | round | variable | homogenous | S | 178±5.17 (208) | 226 |
| 1–C | EC | ++ | ++ | ++ | ++ | polymorphous | intense | homogenous | A | 271±4.13 (257) | 345 |
| 1–D | P | ± | ± | ± | ± | round | intense | homogenous | A | 123±2.71 (121) | 156 |
| 1–E | Alike | + | – | – | – | round | intense | homogenous | S | 177±2.81 (163) | 225 |
| 1–F | ECL | ++ | – | – | – | round ellipsoidal | intense | heterogenous | P large | 372±9.68 (136) | 473 |
| 2–A | G | – | ++ | – | – | round | variable | heterogenous | P | 240±9.53 (68) | 305 |
| 2–B | I | – | – | + | + | round | intense | homogenous | S | 173±5.28 (48) | 220 |
| 2–C | K | – | – | ± | ± | round polygonal | intense | homogenous | A | 176±3.85 (123) | 224 |
| 2–D | S | – | – | ± | + | round | intense | homogenous | P | 214±8.88 (64) | 272 |
| 2–E | X | + | – | – | – | egg-shaped polygonal | variable | homogenous | A | 205±3.07 (301) | 261 |
| 2–F | ? | + | – | – | – | polymorphous | intense | homogenous | A | 192±2.96 (223) | 244 |

F = fundus; PA = pyloric antrum; D = duodenum; J = jejunum
++ many; + few; ± very few; – absent
P = present; S = sometimes present; A = absent

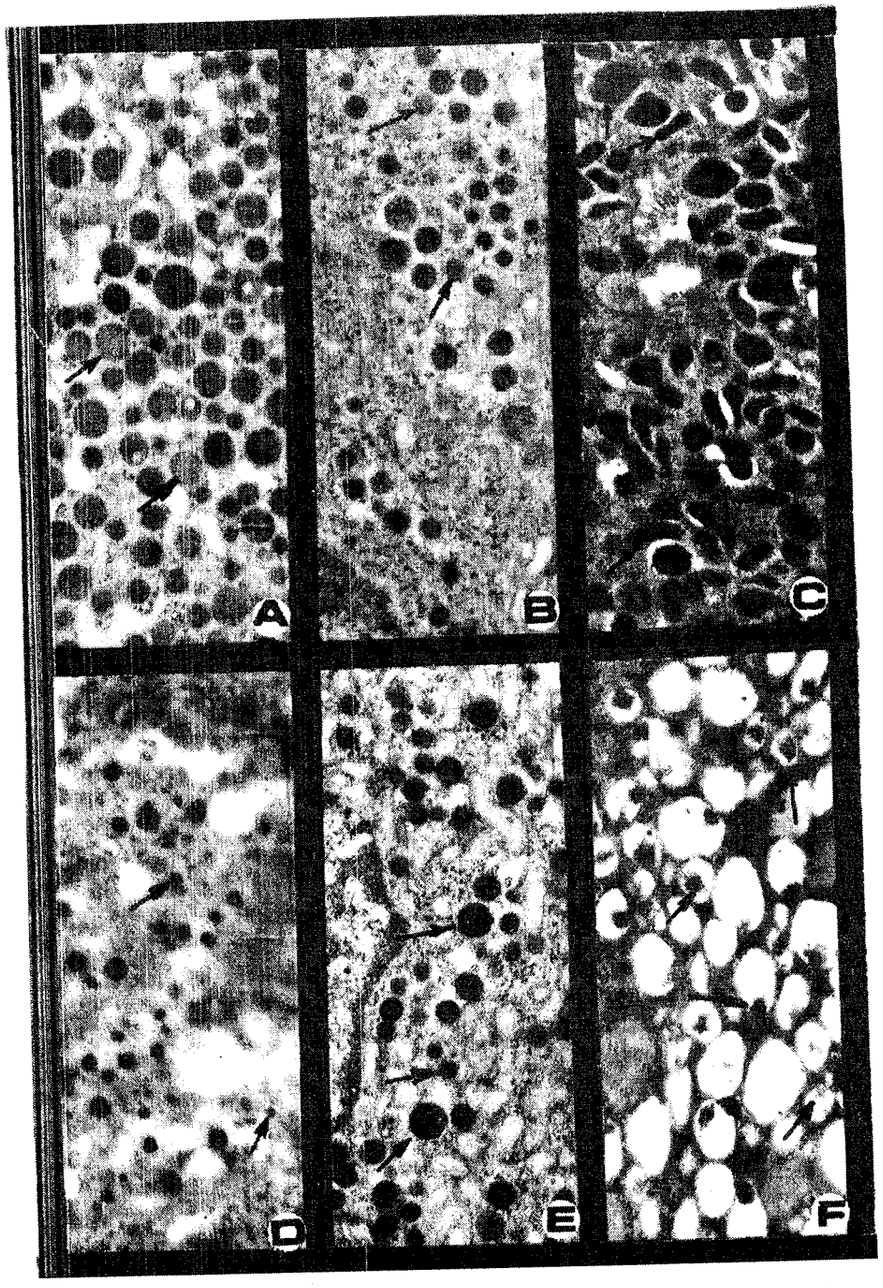


Fig. 1. – A–D cell; all studied gut regions; moderate-slight (arrows) osmiophily, round shapes (266 nm).
B – $D_1$ cell; all studied gut regions; moderate-slight (arrows) osmiophily, round shapes (226 nm).
C – EC cell; all studied gut regions; polymorphos (arrows) shapes (345 nm).
D – P cell; all studied gut regions; intense osmiophily, round small (arrows) shapes (156 nm).
E – A-likl cell; stomach fundus; intense osmiophily, round (arrows) shapes (225 nm).
F – ECL cell; stomach fundus; large halo. heterogeneous (arrows) content (473 nm).

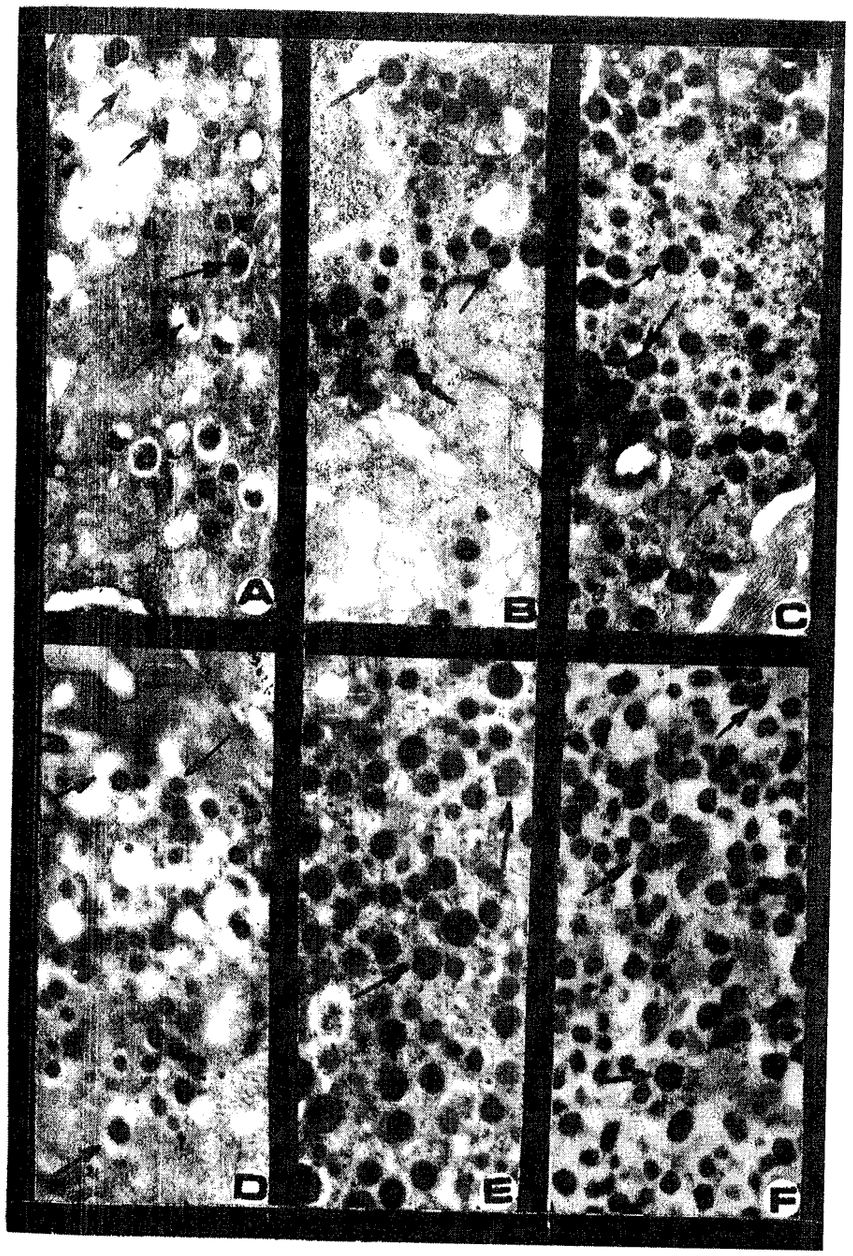


Fig. 2. – A–G cell; pyloric antrum; halo, heterogenous (arrows) content (305 nm).
B – I cell; intestine; intense osmiophily, round (arrows) shape (220 nm).
C – K cell; intestine; intense osmiophily, some polygonal (arrows) profiles (224 nm).
D – S cell; intestine; halo (arrow), intense osmiophily, round shape (272 nm).
E – X cell; stomach fundus; intense osmiophily, some polygonal (arrows) profiles (261 nm).
F – ? cell; stomach fundus; intense osmiophily, polymorphous shapes, some hexagonal, pentagonal (arrows) profiles (244 nm).

*Table 2*

Statistical analysis of sizes of secretory granules contained in endocrine cell types identified in mouse gastrointestinal mucosa. Comparison to other mammalian species

| Cell type | Measured diameter (mean±SEM) [mm] | Calculated diameter (nm) | Significance of difference compared to cell type: | Rabbit stomach Lefranc [13] (nm) | Calves abomasum Domeneghini [4] (nm) | Sheep abomasum Oonmori [20] | | Cat intestine Lefranc [17] (nm) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | measured (nm) | calculated (nm) | |
| P | 123±2.71 | 156 | I-SE*($p < 0.001$) | 90 | | | | |
| I | 173±5.28 | 220 | D -NS**; ?-SE ($p < 0.01$); X-SE($p < 0.001$) | | | | | 300 |
| A-like | 177±2.81 | 225 | K-NS; ?-SE ($p < 0.001$) | | 149±30 | | | |
| K | 176±3.85 | 224 | D -NS; ?-SE($p < 0.101$); X-SE($p < 0.01$) | | | | | 320 |
| D1 | 178±5.17 | 226 | ?-NS; X-SE($p < 0.001$) | 125 | 124±22 | 169±32 | 215 | |
| ? | 192±2.96 | 244 | X-SE ($p < 0.01$); D-SE($p < 0.001$) | | 148±33 | | | |
| X | 205±3.07 | 261 | S-NS; G-SE($p < 0.001$) | 225 | 169±39 | 302±56 | 384 | |
| D | 209±3.82 | 266 | S-NS; G-SE($p < 0.01$); EC-SE($p < 0.01$) | 255 | 169±38 | 272±46 | 346 | |
| S | 214±8.88 | 272 | G-NS; EC-SE($p < 0.001$) | | | | | 250 |
| G | 240±9.53 | 305 | EC-SE($p < 0.01$); ECL-SE($p < 0.001$) | | | 267±40 | 340 | |
| EC | 271±4.13 | 345 | ECL-SE($p < 0.001$) | 250 | 110±33 | 206±44 | 262 | |
| ECL | 372±9.68 | 473 | EC-($p < 0.001$) | 285 | 153±50 | 261±55 | 332 | |

*SE = significant difference; **NS = not significant difference



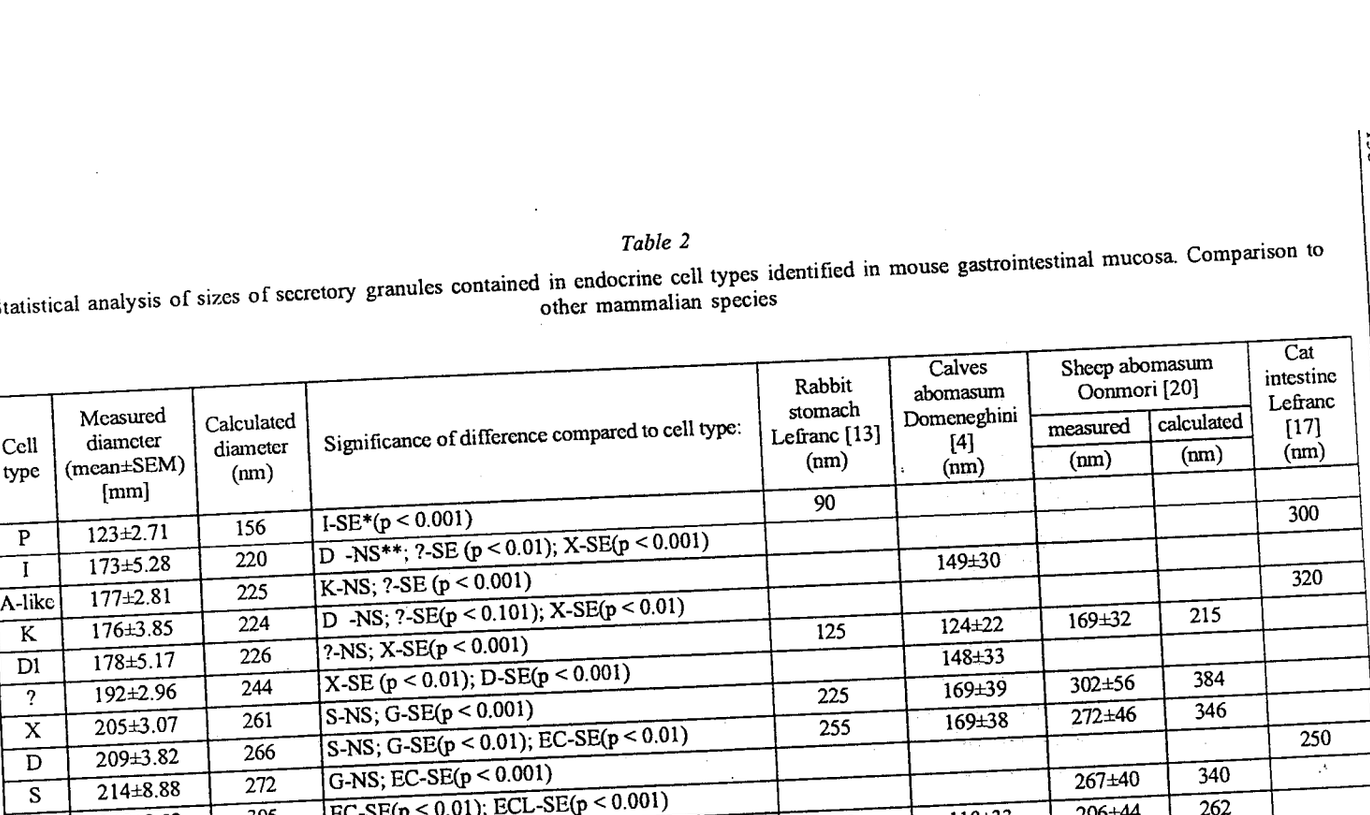


Fig. 3. – Frequency distributions of the secretory granule diameters in twelve endocrine cell types identified in mouse gastrointestinal mucosa.

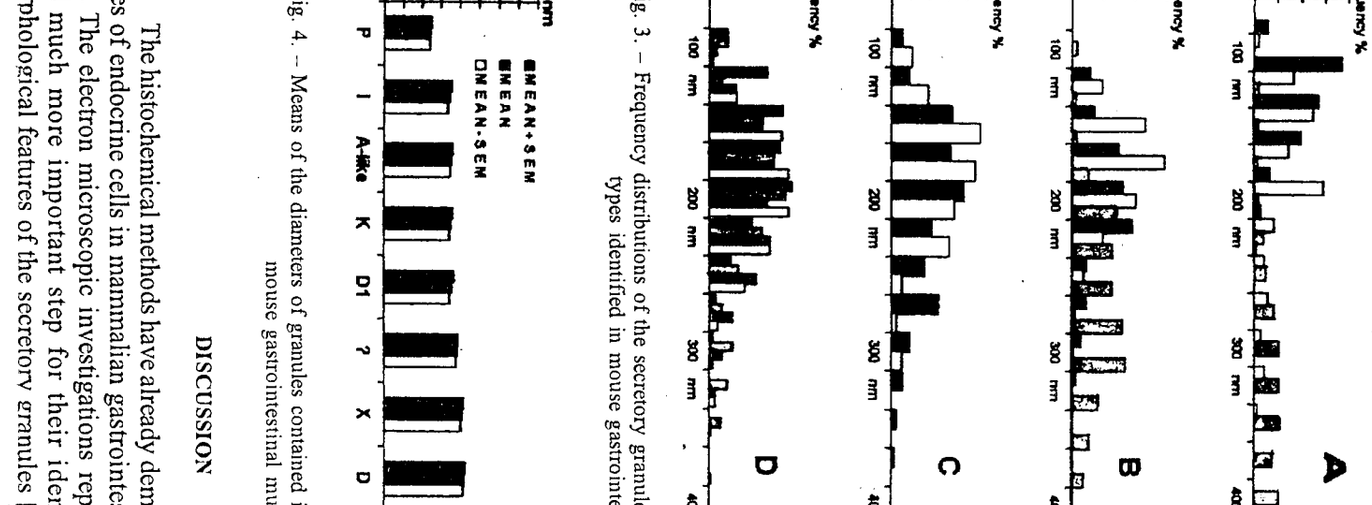


Fig. 4. – Means of the diameters of granules contained in endocrine cell types identified in mouse gastrointestinal mucosa.

## DISCUSSION

The histochemical methods have already demonstrated the presence of various types of endocrine cells in mammalian gastrointestinal tract [2, 3, 5, 6, 9, 15, 16, 25]. The electron microscopic investigations represented undoubtedly a further and much more important step for their identification based on different morphological features of the secretory granules [2, 4, 7, 10, 11, 12, 24, 26, 27].

The present work describes twelve enteroendocrine cell types and their distribution in the stomach and along the upper intestine of mouse. Most of them (11 types) have been also found in other mammalian species [1, 3, 6, 7, 12, 13, 15, 17, 20, 27] and are included in the current international classification [26] of cells belonging to the gastro-entero-pancreatic (GEP) endocrine system.

In comparison with other mammalian species, the sizes of secretory granules of enteroendocrine cell types in mouse varied between the same ranges of 100-350 nm (Table 2). The granules of D cells showed greater sizes compared to those of $D_1$ cells, in the species presented in Table 2.

Special attention was paid to the identification of X cells, distributed exclusively not only in the mouse stomach fundus, but also in the same region of gastrointestinal tractus in cat [17], rat [6], rabbit [3, 15, 16], and human [7, 27]. Due to the ultrastructural peculiarities of the secretory granule population (variable osmiophily, pentagonal, hexagonal, but most round profiles) (Fig. 2E) the identification of this cell type was difficult.

Finally, we detected in the stomach fundus an endocrine cell type (Fig. 2F), showing similar ultrastructural features with those reported by Domeneghini (1985) [4] in calves abomasum. According to our opinion it could represent a new cell type, still not included in the international nomenclature of GEP endocrine system cells. On this line, the further demonstration of its presence in the gastric mucosa of other mammalian species is strongly requested.

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# A NEW METHOD FOR PURIFICATION OF SEMINOLIPID AND LYSOSEMINOLIPID FROM SPERM CELLS AND TESTIS

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Seminolipid (1-0-alkyl-2-0-acyl-3-[β-D-3-sulfogalactopyranosyl]-sn-3-glycerol) was purified from rat testis and from boar spermatozoa and testis. The following methods were used: extraction with chloroform-methanol mixtures, Folch partition, adsorption chromatography on Florisil and ion-exchange chromatography on DEAE-Sephadex A-25. Usually, in this stage seminolipid gave one band at thin layer chromatography in three solvent systems: acidic, neutral and basic. Otherwise, an adsorption chromatography on silicic acid was accomplished. Lysoseminolipid (1-0-alkyl-3-[β-D-3-sulfogalactopyranosyl]-sn-3-glycerol) was prepared by mild alkaline hydrolysis of seminolipid. The product was separated from the cleaved fatty acid and from other compounds by adsorption chromatography on silicic acid; a product giving a unique spot by thin layer chromatography has been obtained.

Sulfated glycolipids are a class of acidic glycolipids containing one or two sulfate esters on their oligosaccharide chains. Till now, more than 20 sulfated glycolipids have been isolated from various tissues of vertebrates, echinoderms and microorganisms and their structures were elucidated (18).

Sulfation turns neutral glycolipids into anionic species. The enzymes that add sulfate (10) to glycoconjugates appear to be exclusively located at the luminal side of Golgi stacks, whereas kinases operate at many different subcellular sites. Generally, therefore, sulfated glycolipids are either secreted by the cell or inserted into the extracellular leaflet of the plasma membrane. On the other hand, phosphates containing lipids, as for example phosphatidylinositol phosphates, were found predominantly at the cytosolic side of the plasma membrane or at other intracellular sites (18).

Boar testis, rat testis and boar spermatozoa constitute well established sources of seminolipid (1, 4, 7). Moreover, seminolipid has been found in the rat brain (6).

The present study describes the isolation of seminolipid and preparation of seminolipid by using ion-exchange and adsorption chromatography. (IUPAC nomenclature rules indicated by Ledeen and Yu (8) and by Hori and Sugita (3) as well as the nomenclature rules proposed by the cited authors have been used).

## MATERIALS AND METHODS

*Materials*. Plates for thin layer chromatography and silicic acid for column chromatography were purchased from Merck (Germany), Florisil from Floridin

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Co., (U. S. A), DEAE-Sephadex A-25 from Pharmacia (Sweden). Standard glycolipids (GalCer and GalCer $I^3$ sulfate) have been purified from the rat brain (5). Boar sperm and boar testis were provided by Peris Institute, Romania. Rats of R strain were used as a source of testes.

*Methods*. Boar testis, boar spermatozoa or rat testis were extracted repeatedly with warm chloroform-methanol-water mixtures. Lipidic mixture was partitioned by mixing with 0.8% KCl solution (8). Lower phase lipids were chromatographed on Florisil by using increasing concentrations of methanol in chloroform, as eluant. The collected fractions were monitored by thin layer chromatography. The fractions containing seminolipid were mixed and submitted to ion-exchange chromatography on DEAE-Sephadex A-25. Elution of seminolipid was accomplished with increasing concentrations of sodium acetate in methanol, the fractions being monitored by thin layer chromatography. Excess sodium acetate was removed by partition in chloroform-methanol-water mixtures, upper phase being discarded.

The following solvent systems were used at thin layer chromatography: I, chloroform-methanol-water (60:25:4, v/v); II, chloroform-methanol water-concd. ammonia (70:30:3:1, v/v;) III, chloroform-methanol-acetone-acetic acid-water (10:2:4:2:1, v/v;). The plates were visualised by spraying with orcinol followed by heating (5).

Seminolipid was submitted to mild alkaline hydrolysis followed by neutralysation and partition in chloroform-methanol-water mixtures. Purification of lysoseminolipid was accomplished by silicic acid chromatography on a column washed with increasing concentrations of methanol in chloroform.

## RESULTS AND DISCUSSION

Chromatography of sperm lipids from lower phase on Florisil removed phospholipids as well as neutral lipids (cholesterol, cholesterol esters, triglycerides etc.). At the same time, derivatives of seminolipid, as for example lysoseminolipid and desulfoseminolipid, could be eluted from the column; both were noticed at least in traces. From boar testis they could even be separated.

The use of ion-exchange chromatography on DEAE-Sephadex A-25 improved appreciably the quality of separation of sulfated compounds. Seminolipid that had been purified by this method gave usually one band at thin layer chromatography in three solvent systems (Fig. 1).

1-0-Alkyl bond of seminolipid is resistant to alkaline attack, so that mild alkaline hydrolysis proved an excellent method for preparation of lysoseminolipid from seminolipid. Further purification of lysoseminolipid was accmplished by silicic acid column chromatography, a pure compound being obtained (Fig. 1).

The sulfated glycero-glycolipid from rat brain consisted of a mixture of diacyl- and 1-0-alkyl-2-0-acyl-3-[β-D-3-sulfogalactopyranosyl]-sn-3-glycerol (6). Both sulfated glycero-glycolipid and sulfatides were synthetized most actively at the age of 18 days, like known components of myelin of the central nervous system (6).

The following sulfoglycosphingolipids have been found in rat kidney: GalCer $I^3$-, LacCer $II^3$ -sulfate, Gg3Cer $II^3$, $III^3$ -bis-sulfate (15, 17), Gg3Cer



Fig. 1. – Thin layer chromatography of seminolipid (lane one on plates a, b and c), and lysoseminolipid (lane two on the plates a, b and c). Plates a, b and c were migrated with solvent mixtures I, II and III, respectively.

$II^3$ -sulfate (14,16). Galcer $I^3$-sulfate and LacCer $II^3$-sulfate have been found also in porcine plasma (2). It was found that hog gastric mucosa contained GalCer $I^3$-sulfate, LacCer $II^3$-sulfate, Ga $^3$Cer $III^3$-sulfate, nLc4Cer $III^6$-sulfate (12, 13). By using chemical and immunological techniques, the structure of the spleen sulfolipid was confirmed as being GalCer $I^3$-sulfate; the respective lipid was localized in the spleen granulocytes (11). A new sulfated glycosphingolipid, Gb5Cer $V^3$-sulfate, has been isolated and characterized from human kidney (9).

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# OF GLYCOSPHINGOLIPIDS FROM LOW DENSITY LIPOPROTEIN OF NORMAL HUMAN

DUMITRU-PETRU IGA, SILVIA IGA

Low density lipoprotein fraction from normal human was submitted concomitantly to chloroform-methanol extraction and partition. Lipids from lower phase were analyzed by this layer chromatography; three compounds were found giving positive reaction to orcinol. Two of them moved in the region of gangliotetraosylceramide and the third in the region of monohexosylceramide. Upper phase lipids were submitted to mild alkaline hydrolysis and then dialysed against distilled water. The residue obtained by vacuum evaporation of non-diffusible material was dissolved in chloroform-methanol 1/1 (v/v) and submitted to thin layer chromatography; a ganglioside comigrating to ganglioside GM3 was identified by positive reaction to resorcinol. A compound comigrating to LacCer has been obtained by acidic desialylation. Concentration of lipid bound sialic acid in LDL has been estimated to 3.8 μg/mg protein.

The cholesterol required for making new membranes is taken up by many animal cells through receptor-mediated endocytosis. Blocking of cholesterol uptake leads to its accumulation in the blood and formation in blood vessel walls of atherosclerotic plaques becomes very probable.

Most cholesterol is carried in the blood in a bound state to protein in the form of particles known as low-density lipoproteins (LDL) (1). Chemical composition of LDL concerned especially cholesterol, cholesterol esters, triacylglycerol and phospholipids (4,12).

The aim of present paper was to investigate the type and the amount of glycosphingolipids (GSL) from LDL of normal human.

## MATERIALS AND METHODS

*Materials*. GalCer with normal fatty acids was purified from rat brain, LacCer was provided by Dr. G. Schwarzmann (Bonn University, Germany), gangliosides GM4 and GM3 were purified from chicken brain. Thin layer plates were from Merck (Germany).

*Methods used.* Low density lipoprotein was separated from blood plasma of normal human by gradient centrifugation (1,12). LDL was then submitted concomitantly to extraction with chloroform-methanol mixtures and the two phases were processed separately. Lipids from lower layer were submitted to thin layer chromatography while the lipids from upper layer were studied in natural state as well as after acidic desialylation.

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The following solvent systems were used at thin layer chromatography: I, chloroform-methanol-water (60:25:4, v/v); II, chloroform-methanol-concd. ammonia-water (12:8:1:1, v/v); III, chloroform-methanol-concd. ammonia-water (22:18:1:3, v/v); IV, chloroform-methanol-acetone-acetic acid-water (10:2:4:2:1, v/v). The plates were visualised by spraying with orcinol for glycolipids, resorcinol for gangliosides and phosphomolybdenic acid for all lipidic compounds, followed in every case by heating.

Acidic desialylation was accomplished by solving the probes in 0.05 M HCl followed by heating at 80°C for 1 hr. The probes were then neutralized and partitioned in chloroform-methanol-water mixtures.

## RESULTS AND DISCUSSION

Three compounds giving positive reaction to orcinol were evidenced in lower phase lipids of LDL (Fig. 1). Two of them migrated in the region of gangliotetraosylceramide and the third in the region of GalCer.

A compound giving positive reaction to resorcinol and orcinol was evidenced by submitting lipids from upper phase to thin layer chromatography (Fig. 2). Gangliosides give positive reaction to resorcinol due to sialic acid and their positive reaction to orcinol is due to the presence of neutral sugars (Glc, Gal). The compound giving positive reaction to resorcinol comigrated with GM3 ganglioside in three solvent systems (II, III and IV) (Figs. 3 and 4).

By acidic desialylation the compound giving positive reaction to resorcinol disappeared and a compound giving positive reaction to orcinol but comigrating with LacCer was evidenced (Fig. 4).

A great diversity of glycosphingolipids (GSL) have been found in human plasma and its lipoproteins: GlcCer, GalCer, LacCer, Gb3Cer, Gb4Cer (9, 11, 13).

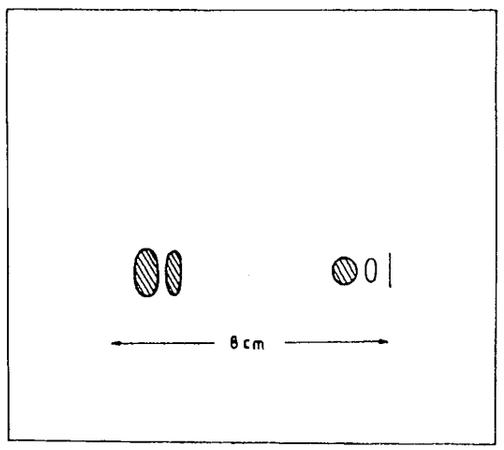
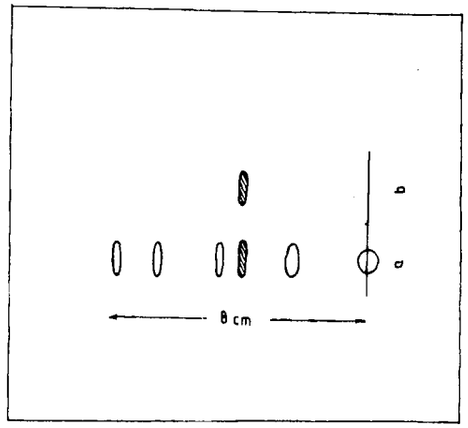



Fig. 1. – Thin layer chromatography of lower phase lipids from LDL. Migration with solvent I, visualisation with orcinol.



Fig. 2. – Thin layer chromatography of upper phase lipids from LDL. On both lanes it was applied the same amount of lipids. Lane a was visualised with orcinol and lane b with resorcinol. Migration with solvent III.



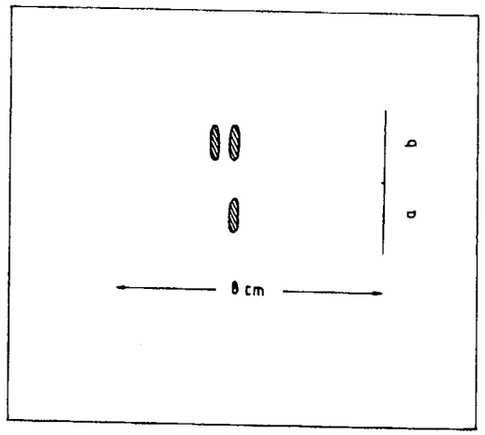
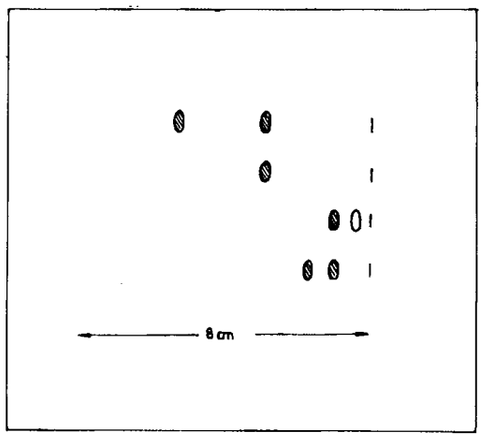




Fig. 3. – Comigration of the ganglioside from human LDL with GM3 ganglioside. Migration with solvent III, visualisation with resorcinol. (The same result was obtained by elution with solvent II).

Fig. 4. – Comigration of asialoderivative from human LDL with LacCer. Lane 1, standards: GM4 and GM3; lane 2, lipids from upper phase; lane 3, LDL lipids from upper phase after desialylation; lane 4, standards: GalCer and LacCer. Migration with solvent IV, visualisation with resorcinol.



Some authors reported on increased levels of GSL in serum: an increase of GlcCer in Gaucher patients and an increase of Gb3Cer in Fabry patients (10). There are also some reports on elevated levels of plasma GSL in patients with familial hypercholesterolemia (2, 3). However, the mechanism of the increase in GSL concentration remains to be solved.

Watanabe hereditable hyperlipidemic rabbit (WHHL rabbit) is an adequate animal model for human familial hypercholesterolemia. Hara and Taketomi (4) analyzed for the first time GSL in serum and lipoproteins from WHHL rabbit. It was found that the major GSL of chylomicrons (CM), very low density, low density and high density lipoproteins contained sulfatide as a major GSL: 12 nmol/$_{\mu}$mol total phospholipids in CM, 19 nmol/$_{\mu}$mol phospholipids in VLDL, 18 nmol/$_{\mu}$mol phospholipids in LDL and 14 nmol/$_{\mu}$mol phospholipids in HDL. Also, other minor GSL such as GlcCer, GalCer, GM3 ganglioside, LacCer and Gb3Cer have been found (4).

The concentration of sulfatide in WHHL rabbit serum is much higher than that in normal rabbit serum (121 nmol/ml versus 3 nmol/ml). Fatty acids of the sulfatides comprised nonhydroxy fatty acids (C22, 23, and 24) as well as hydroxy fatty acids (about 10%). Long chain bases of the sulfatides comprised (4E) – sphingenine and a significant amount of 4D-hydroxysphinganine (about 10%). Moreover, sulfatides in the liver and small intestine (where serum lipoproteins are produced) from normal and WHHL rabbit were estimated to be 260 nmol/g liver in WHHL rabbit, 104 nmol/g liver in normal rabbit, 99.6 nmol/g small intestine in WHHL rabbit and 31.2 nmol/g small intestine in normal rabbit. Ceramide portions of the sulfatides in the liver were composed almost exclusively of (4E)-sphingenine and nonhydroxy fatty acids, while those in the small intestine were especially composed of 4D-hydroxysphinganine and hydroxy fatty acids. The following conclusion was drawn from such results: the sulfatides of serum lipoproteins are mostly derived from the liver (90% of the total), and the other sulfatides (10% of the total) might be derived from the small intestine.

The following GSL have been found in human jejunum: ceramide monohexoside (GalCer, GlcCer), ganglioside GM3 and sulfatide (6). On the other hand, GlcCer is the predominant ceramide monohexoside in the human jejunum. 2-Hydroxylated fatty acids in the total fatty acids of human jejunum sulfatides amounted to 24.9% and in GlcCer to 36% (6).

Kiguchi et al (5) have analyzed the amounts and types of GSL from human peripheral blood lymphocytes, monocytes and granulocytes isolated by countercurrent elutriation. The highest amount of neutral GSLs (109 $_{\mu}$g/$10^8$ cells) was found in granulocytes, and considerably less found in monocytes (11 $_{\mu}$g/$10^8$ cells) and lymphocytes (4 $_{\mu}$g/$10^8$ cells).

Treatment of patients having hypercholesterolemia with heparin induced extracorporeal LDL precipitation resulted in an almost selective removal of lipid-bound sialic acid carried on LDL (8). The total content and pattern of gangliosides were determined in the unfractionated serum of healthy human adults and in isolated lipoproteins. The total content of lipid bound sialic acid was 10.5 nmol/ml serum. The ganglioside profile consisted of >10 different components. The major ganglioside was GM3, followed by GD3, GD1a, GM2, GT1b, sialosyllactoneotetraosylceramide, GD1b, GQ1b and four additional gangliosides. Approximate 98% of the human serum gangliosides were transported by serum lipoproteins predominantly by LDL (66%) followed by HDL (25%) and VLDL (7%). The quantitative distribution of individual gangliosides in VLDL and LDL was almost the same as that in the unfractionated serum; some differences existed with the ganglioside profile in HDL (7).



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# MODULATION OF THE PROLIFERATIVE RESPONSE OF T PERIPHERAL LYMPHOCYTES BY PVH NUCLEUS

V. P. HEFCO, ANA OLARIU and F. GHEORGHIU

Lesion and isolation of the hypothalamic paraventricular nucleus (PVH) induce lymphocytopenia and significant increase of the T lymphocytes response from the circulating blood, to the phytohemagglutinin-M (PHA-M). Fronto-lateral isolation of the medial hypothalamic zone, including the PVH nucleus, induces lymphocytopenia and a significant decrease of the peripheral lymphocytes proliferation. In conclusion, PVH nucleus inhibits the proliferative response of the blood lymphocytes, mainly via the activation of the peripheral sympathetic system and partially via its neurosecretions.

*Key words*: PVH nucleus, sympathetic nervous system, peripheral blood, proliferative response.

The PVH nucleus plays a major role in organism homeostasis, through integration of the nervous and endocrine signals. The parvocellular component of the PVH nucleus secretes some neuropeptides: corticolliberin (CRH)(16, 20), thyroliberin (TRH)(20), peptide HI-27 (PHI-27)(19), vasoactive intestinal peptide (VIP)(20), somatostatin, dopamine and several endogenous opioid peptides (20), neurosecretions which control the function of adenohypophysis. The PVH nucleus is also connected to the autonomic nervous system. Thus, it has connections with the sympathetic preganglionary neurons from the thoracic region of the spinal cord as well as with the dorsal motor nucleus of the vagal nerve from the brain stem (1, 22).

In immune homeostasis, a special importance has the functional response of the T lymphocytes. T helper lymphocytes initiate, while T suppressor ones, participate in regulation of the cellular immune response. On the other hand, in immune homeostatic mechanisms, the central nervous system (CNS) plays an important role. Indeed, as early as 1945, Baciu has studied the role of CNS in regulation of immune response (2). Using different methods to investigate the involvement of the hypothalamic vegetative nervous centers, he has demonstrated that nervous centers from the tuberal area and from the posterior hypothalamus in connexion with a preoptic, anterior and lateral hypothalamic area are involved in the regulation and integration of the immune response (3). Moreover, a series of neurohormones, among which those secreted by the PVH nucleus, too, may influence the number and/or functional response of the T lymphocytes (4, 8).

Because of the essential role of PVH nucleus in organism homeostasis, we investigated the mechanisms by which the PVH nucleus influences the organism defence function. In this way, we have lesioned or isolated PVH, either alone, or together with the medial sympathetic hypothalamic area, including also the hypophysiotropic area, and we have determined the circulating lymphocytes number and the proliferative response to PHA-M of lymphocytes from peripheral blood.

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## MATERIALS AND METHODS

Male Wistar rats weighing 200-300 g at the beginning of the experiments were used. They were housed four per cage and they had free access to food (McCollum standard diet) and water.

Using the stereotaxic method, the PVH nucleus was either mechanically lesioned (10), isolated alone, or isolated at the fronto-lateral region of the hypothalamus (11). In the last case, the anterior, dorsal and lateral connections of medial hypothalamic area have been sectioned. In shame operated rats, the knife was lowered 5 mm below the skull surface, without subsequent rotations. The histological control of the interventions was performed according to the method of Guzman-Flores (9).

21 days after the surgery, the blood was collected from retroorbital venous plexus in animals under ether anaesthesia. The lymphocytes number was determined from leukocytary formula. The proliferative response of the lymphocytes to PHA was tested "in vitro", using whole heparinized blood. Thus, in centrifuge tubes (Corning TM, FOB Sigma), 5 ml of HAM's medium (Biochrom KG Berlin, Germany), 75 µl PHA-M (Biochrom KG Berlin, Germany) and 75 µl whole heparinized blood were mixed. The tubes thus prepared have been kept at 37° C. After 72 hours, the samples were centrifuged at 1,200 rpm, for 10 minutes and the supernatant was removed. Smears were prepared from the sediment, and stained according to the Giemsa method. By microscopic examination we determined the index of the blastic transformation, expressed as the number of lymphoblasts per 100 lymphocytes counted.

The results were analysed statistically using Student's t test.

## RESULTS

PVH lesion (PVHL), PVH isolation (PVHI) and fronto-lateral isolation (FLI) induce significant decrease of the lymphocytes number (Table 1), more pronounced decreases have been recorded in FLI animals.

The test of blastic transformation of the T lymphocytes from peripheral blood, (Fig. 1), indicates an increased response for PVHL and PVHI groups, while FLI induces a decrease in the proliferative response when it is compared with SO group.

*Table 1*

Changes of the number of the circulating lymphocytes after PVH lesion, PVH isolation and frontolateral isolation. Number of rats in each group was five. Values are expressed as mean ± SE. N = normal; SO = sham-operated; PVHL = PVH nucleus lesioned; PVHI = PVH nucleus isolated; FLI = frontolateral isolation

| Groups | N | SO | PVHL | PVHI | IFL |
|---|---|---|---|---|---|
| Number of circulating lymphocytes | 9.839 ± 274 | 9.562 ± 165 | 7.338 ± 298<br>$p < 0.001$ | 8.064 ± 96<br>$p < 0.001$ | 5.977 ± 262<br>$p < 0.001$ |



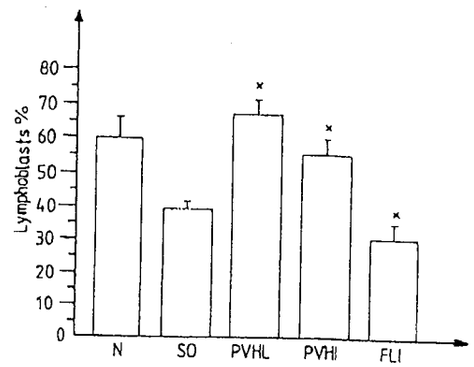


Fig. 1. – The proliferative response of T peripheral lymphocytes to PHA-M. Values are expressed as the mean ± SE of five rats. N = normal; SO = sham operated; PVHL = PVH nucleus lesioned; PVHI = PVH nucleus isolated; FLI = fronto-lateral isolation. Signiicant variations are indicated by "*"

## DISCUSSION

Our data showed that mechanical lesion and isolation of the PVH nucleus, as well as fronto-lateral isolation of the medial hypothalamus, including the PVH nucleus, induce a significant decrease of the circulating lymphocytes number. The proliferative response of the T lymphocytes to PHA is increased in PVHL and PVHI group, and decreased after FLI of the medial hypothalamic region.

The neuropeptides secreted by the PVH nucleus: CRH (16, 20), VIP (20), somatostatin and certain morphin-like peptides, have both an endocrine and immunomodulatory role. It is known that somatostatin, VIP, beta-endorphin, and glucocorticoids have an inhibitory effect on lymphocytes response to different mitogens (4, 7, 18). In our experiments, lesion of the PVH nucleus has increased the response to PHA, probably because the reduction of somatostatin, beta-endorphin and VIP secretion by the PVH nucleus, after its lesion. Isolation of the PVH nucleus, when the secretion of these peptides is not affected, determines, too, a significant increase of the response, but less ample as compared with PVHL. These results, show that the peptides secreted by PVH nucleus can have a low inhibitory role. Moreover, FLI -without modifying the secretion of the hormone already mentioned, induces a significant decrease of the response, which emphasizes the idea that there are also other inhibitory pathways of the proliferative response.

The PVH nucleus is connected also with the autonomic nervous system, and it has a sympathetic nature (17). Thus, its lesion reduces the activity of the splanchnic nerve and enhances the vagal activity (22), and its stimulation increases blood pressure, via activation of the sympathetic system (17).

Sympathetic pathways are involved in modulation of many lymphocytes functions from the lymphoid organs or peripheral circulation (5, 6, 12,). According to some data, the proliferative response of the T lymphocytes from the spleen is modulated by signals transmitted through the splenic nerves (14, 21). Studying the effect of stress on peripheral lymphocytes response, Cunnick have observed that adrenalectomia prevents suppression of this response to stress, effect probably mediated by glucocorticoids and catecholamines (21). The action of catecholamines

involves maybe the ß-adrenergic receptors on T lymphocytes, because their activation decreases the expression of IL-2 receptors of the T lymphocytes to IL-2(15).

In our experiments, PVHL shows a significant increase of the blastogenic response. Because stimulation of the PVH nucleus increases the catecholamine secretion by adrenal medulla (17), lesion of this nucleus, accompanied by the decrease of the splanchnic nerves activity, is expected to induce, too, a decrease of the catecholamines level. As catecholamines inhibit the T lymphocytes proliferation, the increase of the response to PHA, observed in our experiments, might be determined by diminution of the catecholamines level. In PVHI group, when are sectioned all nervous links of the nucleus, including those with the sympathetic preganglionary neurons, we have recorded also a significant increase of the blastogenic response. On the other hand, in FLI group, the response to PHA decreases significantly. In this case, the dorsal longitudinal fascicle of Schütz, the main output of the sympathetic hypothalamus, is still functional, so, PVH nucleus may continue to transmit signals to the peripheral sympathetic nervous system, and to inhibit – via catecholamines – the proliferative response.

Determination of the lymphocytes number for all studied groups shows a significant decrease. In 1983, Crary showed that administration of epinephrine to humans induced lymphocytosis (14). Other authors have also obtained lymphocytosis, after acute stimulation of the sympathetic activity, like in dynamic exercises or mental stress (13). On having this view, the assertion might be made that lymphocytopenia which we have recorded, as a result of PVHL, and PVHI, might be due to the lowering of the sympathetic tonus. A significant decrease of the lymphocytes number in FLI group cannot be explained, at least for the time being.

In conclusion, the PVH nucleus inhibits the proliferative response of the peripheral T lymphocytes to the action of PHA. Its inhibitory role is exercised both on a nervous and endocrine way, the former being predominant, and manifested by means of the sympathetic nervous system. Therefore, the PVH nucleus assures a sympathetic tonus, inhibiting the T lymphocytes function, and thus controlling both initiation and regulation of the immune response.

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# HEPATIC RECOVERY AT YOUNG RATS. CONSECUTIVELY TO THE INTOXICATION WITH $CCl_4$ DURING LACTATION

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The female rats weighing 200-225 g during lactation were intoxicated with carbon tetrachloride. We studied the effects on their young rats which were suckled. Carbon tetrachloride penetrated through the milk in the body of the young rats and produced liver injuries typical for $CCl_4$. After interruping the intoxication with $CCl_4$ of female rats (mother), we observed the recovery of the liver of young rats.

Carbon tetrachloride is a xenobiotic which can be found in human environment. The toxicity of $CCl_4$ is well-known, being able to induce at liver level diseases which range from steatosis to cirrhosis and cancer (2, 3, 9).

Most lab animals, but especially the rats and mice, are sensible to the action of this xenobiotic. Carbon tetrachloride is frequently used in studies of inducing the hepatic toxicosis, especially at adult animals (3, 4, 8).

The regeneration of the liver after the surgical ablation is very much studied, less studied being the regeneration consecutive to toxicosis. That is why we thought it was interesting that we studied the toxicosis with $CCl_4$ and recovery at the liver level in earlier ontogenetic stages, for instance with young rats during lactation.

## MATERIAL AND METHODS

In our experiments we used female rats, white Wistar weighing 200 ± 25 g being properly kept and fed, as well as their cubs aged one day.

*The first stage*: The mother rats and the young rats sacrificed after 7 days of treatment began when the young rats were only one day old.

We made up two groups:

*The control group*: marked with *M*. Every female from this group received 0.5 ml sunflower oil / 100 g body weight for 7 days running after the birth.

*The group intoxicated with $CCl_4$* marked with *C*. Each female from this group got 0.03 ml $CCl_4$ in 0.5 ml sunflower oil/100 g body weight for 7 days running. The first stage lasted 7 days after the birth of the young rats, after which the first sacrifice followed. In the 2nd stage the mothers and the young rats did not get any treatment and they were sacrificed 12 days after the first sacrifice.

The sacrifice had been done by cervical dislocation. Blood was taken for the determination of transaminases activity GPT and GOT (method Reitman-Frankel) and liver for the histology examination (hematoxylin-eosin staining), histochemical (Sudan black for lipids) and histoenzymological (succinate dehydrogenase/SDH/ and cytochromeoxidase/CyOx/) by usual methods (6).

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## RESULTS AND DISCUSSIONS

*The first stage:* female and the young rats, sacrificed after 7 days of treatment.

*Females:* presented (group C) a liver with modifications typical for the intoxication with $CCl_4$ centrolobular necrosis, steatosis, many ballonized cells (Figs 1, 2).

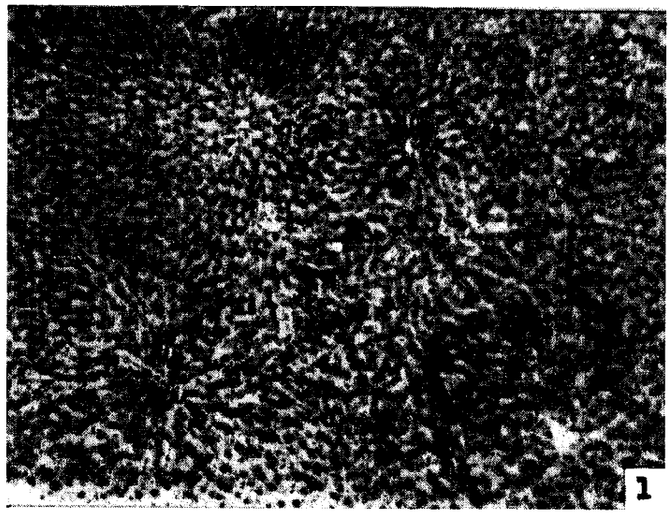

Fig. 1 – Female rats, M group, hematoxylin – eosine staining.

Fig. 2 – Female rats, C group, hematoxylin-eosine staining.



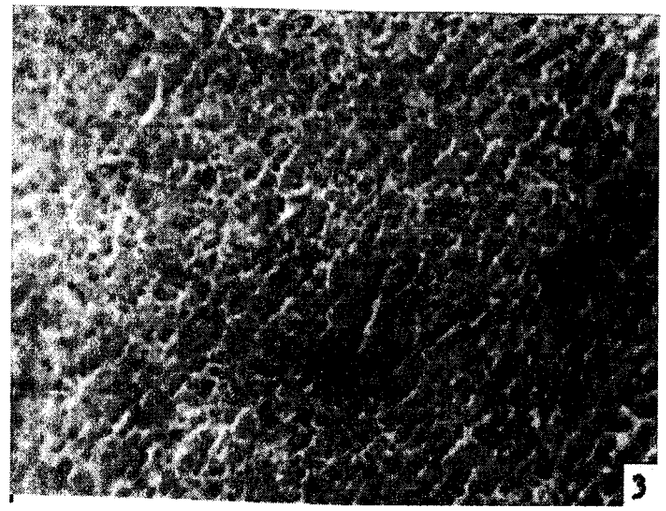

Fig. 3. – Young rats, M group, hematoxylin-eosine staining.

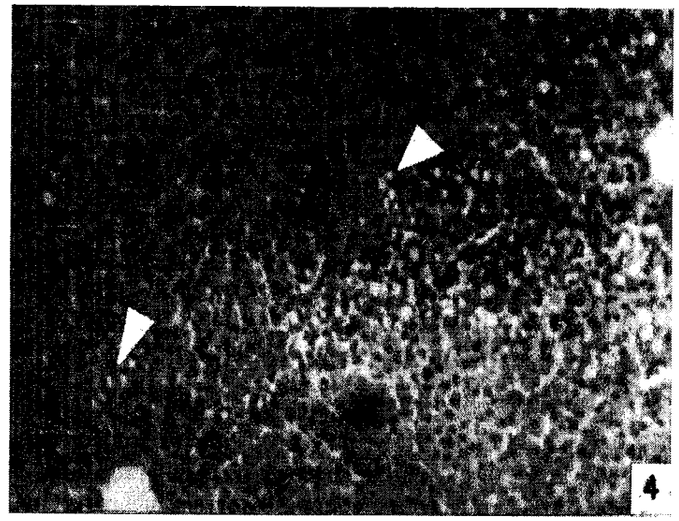

Fig. 4. – Young rats, C group, hematoxylin-eosine staining

*Young rats*: the level of serie transaminases, especially GPT, is higher at the C group with 41%; GOT is not significantly changed (Table 1). The coloration with hematoxylin-eosine characteristic shows, in the liver of young rats, injuries which follow the toxicosis with $CCl_4$ (steatosis, necrosis, ballonized cells) but lower than those of females (Figs 3, 4). The coloration with black Sudan for lipids shows a larger quantity of lipids in comparison with group M. The activity SDH and CyOx does not change.

*Table 1*

I period. The activity of seric transaminase (GPT and GOT) in the $CCl_4$ intoxicated young rats

| | GPT | | GOT | |
|---|---|---|---|---|
| | M | $CCl_4$ | M | $CCl_4$ |
| x | 7.8 | 11 | 43 | 50.9 |
| n | 6 | 6 | 6 | 6 |
| ES± | 0.9 | 0.7 | 1.0 | 7.3 |
| D% | 100 | 141 | 100 | 118.4 |
| t | – | 2.6 | – | 0.9 |
| p | – | >0.05 | – | NS |

*Table 2*

II period. The activity of seric transaminase (GPT and GOT) in the $CCl_4$

| | GPT | | GOT | |
|---|---|---|---|---|
| | M | $CCl_4$ | M | $CCl_4$ |
| x | 15.6 | 22.4 | 36 | 41.5 |
| n | 6 | 6 | 6 | 6 |
| ES | 1.2 | 1.7 | 1.3 | 0.8 |
| D% | 100 | 143 | 100 | 115.3 |
| t | – | 3.54 | – | 2.8 |
| p | – | >0.01 | – | >0.02 |

*The second stage:* young rats sacrificed when they were 19 (12 days after the first sacrifice). The value of seric GPT is 43% higher – at group C in comparison with group M. GPT seric is 15.3% higher – at group C. We cannot see histologic modifications, except some rare necrosis at group C. We did not see any lipidic sediments or any other alterations.

Our results obtained at female rats consequently to the administration of $CCl_4$ for 7 days running confirm the date from literature which describe severe hepatic injuries (1, 7, 8, 10, 11). But the liver of the young rats is also affected both histologically and biochemically (moderate cytolysis, necrosis, ballonizations, steatosis). The modifications were typical for hepatic toxicosis with $CCl_4$ but more moderate than those of the female (mother) rats.

We consider that $CCl_4$ or the active metabolite of this – $CCl_3$ passed in maternal milk and in this way produced significant injuries in the liver of young rats. After 12 days from the first sacrifice, the liver recovered to a great extent from a histological point of view (but the level of GPT is not changed). Stopping the administration of $CCl_4$ indirectly – by maternal milk – allowed the recovery of the liver.



## CONCLUSIONS

Our data prove the possibility that $CCl_4$ or its metabolite – $CCl_3$ may pass in maternal milk and produce typical hepatic injuries at young rats which were suckled (nursed).

The interruption of the administration of $CCl_4$ to mother produces almost a total recovery of the liver of the young rats.

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# BIOELECTRICAL EFFECTS OF SOME POLYOLIC AND POLYPHENOLIC COMPOUNDS IN NORMAL MEDIUM CONDITIONS

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The bioelectric effects of polyacetylated products – pentaacetylglucose, hexaacetylmanitol and hexaacetylsorbitol – have been investigated, on frog striated muscle fibers, comparatively with the base non-acetylated agents – glucose, manitol and sorbitol (5 mg/100 ml normal Ringer). Also, the effects of the polyphenolic fractions (methanolic and ethanolic, 5 mg dry matter/ml normal Ringer) extracted from *Asclepias syriaca* leaves have been studied. The acetylated derivatives induce a stable membrane hyperpolarization (2.64 – 4.27 mV), depending on their nature, higher than that of non-acetylated agents (0.87 – 2.59 mV). The polyphenolic products induce membrane depolarization, ampler for methanolic (22.93 mV) than for ethanolic fraction (14.60 mV).

A series of previous investigations has put into evidence some specific effects of new, polyolic type (acetylated glucidic and polyolic derivatives) and polyphenolic type products (polyphenolic fractions extracted from *Asclepias syriaca*) manifested at cellular level, both upon the cell membrane and membrane phenomena, and upon the cellular metabolism. Positive effects of glucose, manitol and sorbitol acetylated derivatives have been thus observed on the dynamics of leukocytes, seric complement, circulating immune complexes and on the organism defence capacity (1), (4), in parallel with obvious effects on the membrane potential (3), (4) and active transmembranary ionic transport (2); also, intense cytostatic, antitumoral, metabolic and membranary actions of the methanolic and ethanolic fractions from the polyphenolic extracts of the *Asclepias syriaca* leaves, have been evidenced (2), (7), (8).

The present study is devoted to the bioelectrical effects of the above-mentioned products, in a normal physiological medium.

## MATERIAL AND METHOD

The effects induced by polyacetylated products – pentaacetylglucose (PAG), hexaacetylmanitol (HAM), hexaacetylsorbitol (HAS) (5), (6) – on the membrane potential have been studied comparatively with some base, non-acetylated agents – such as glucose (G), manitol (M) and sorbitol (S), in concentration of 5 mg/100 ml normal physiological Ringer solution (NR) and also comparatively with propylenglycol (PG) (0.5 ml %), as a solvent of the acetylated derivatives.

Also, there were followed the bioelectrical effects of the polyphenolic fractions extracted from *Asclepias syriaca* leaves, i.e. the methanolic (MF) and the ethanolic fraction (EF), in a concentration of 5 mg dry matter/ml NR.

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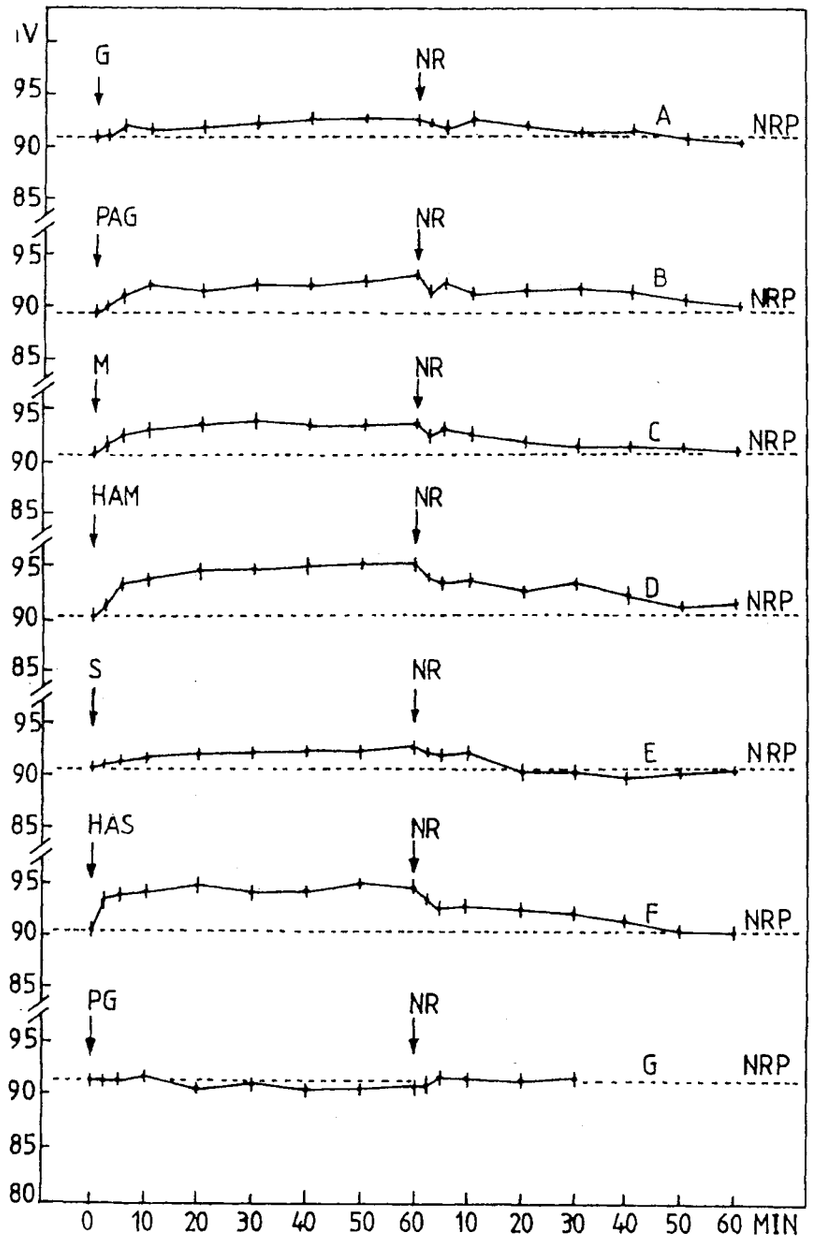


Fig. 1. – The effect of the polyolic acetylated derivatives upon the membrane potential (G = glucose, M = manitol, S = sorbitol, PAG = pentaacetylglucose, HAM = hexaacetylmanitol, HAS = hexaacetylsorbitol, PG = propylenglycol, NR = normal Ringer, NRP = normal resting potential).



The resting membrane potential (RP) of the striated muscular fibers of sartorius frog (*Rana ridibunda* Pall.) muscle has been determined by the method of glass intracellular electrodes, at room temperature. Statistical evaluation employed Student's test.

## RESULTS

Treatment of the striated muscle fibers with the polyols' acetylated derivatives (PAG, HAM, HAS) evidenced a pronounced increase in the values of the membrane resting potential (hyperpolarization) (Fig. 1), the effect amplitude being dependent on the agents' nature. The non-acetylated base products (G, M, S) also showed a hyperpolarizing effect, although much weaker, which makes one assume that acetylation of the products (with 5 or 6 acetyl radicals) causes increase of the specific membranary effects.

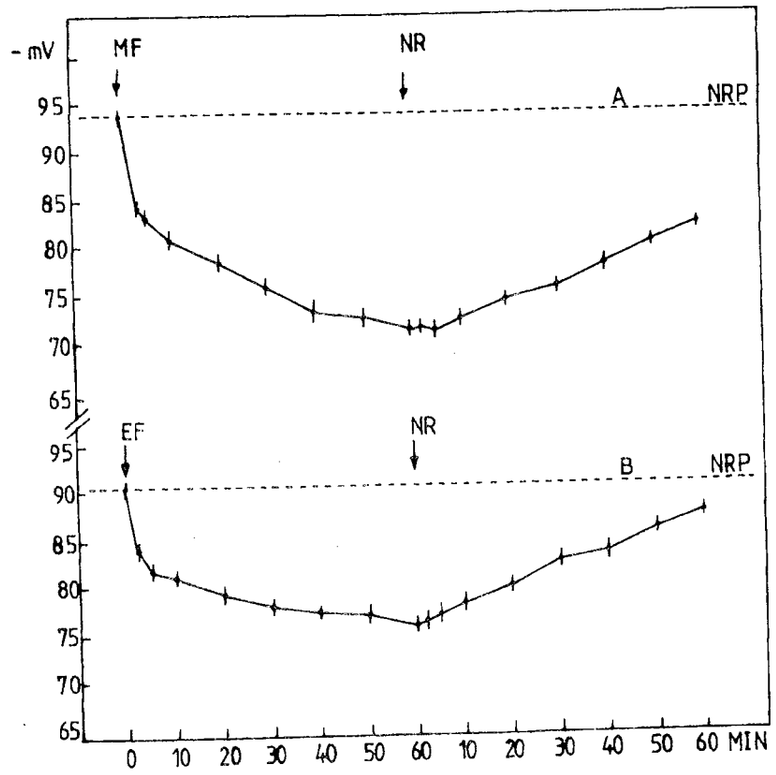


Fig. 2. – The effect of the polyphenolic fractions upon the membrane potential (MF = methanolic fraction, EF = ethanolic fraction).

The amplitude of the hyperpolarization recorded was of 2.81 mV for PAG, 3.57 mV for HAS, 4.27 mV for HAM, 0.89 mV for G, 1.34 mV for S, 2.59 mV for M (Fig. 1). The agents' hyperpolarizing effect is reversible during washing of fibers with a Ringer solution without agents (NR), although quite slow, which assumes a strong bonding of these products in the structure of the muscle fibers membrane.

The effect induced by propylenglycol (PG), the solvent of the acetylated polyolic derivatives, is negligible (i.e. a very weak depolarization).

The action of the polyphenolic fractions (MF and EF) from *Asclepias syriaca* on the membranes of the muscle fibers is nevertheless manifested through a pronounced increase in the values of the RP (depolarization), ampler for MF (22.93 mV) than for EF (14.60 mV) (Fig. 2). Such a depolarizing effect is hardly reversible when the muscular preparations are brought again in NR, which evidences a strong interaction of these agents with the membrane structure.

## DISCUSSIONS AND CONCLUSIONS

The bioelectrical effects specific to the products under study are based on their intcraction with the supramolecular structure of the cell membrane, on the passive and active transmembranary ionic flows as well as on the $Na^+$–$K^+$–ATP–ase's activity.

Thus, the hyperpolarizing effect of the acetylated polyolic derivatives has a multiple determinism.

Acetylation of the base products causes increase in their molecular lipophilicity, which induces their increased interaction with the lipidic membranary structure. Further on, this causes an increased packing degree of the membranary structure, along with its stabilization and production of the membrane fluidity, which influences both the ionic flows and the membrane potential (2–4).

The hyperpolarizing effect is correlated with the observation that such agents stimulate the activity of the $Na^+$–$K^+$–ATP–ase and thus of the active ionic transport, with a correlative modification of the passive ionic flows through the membrane (2), and with a stimulation of the cell metabolism, thus assuring the energy source for the $Na^+$–$K^+$ pump (unpublished data).

A comparison of the acetylated products' effect with that of the non-acetylated base evidences that PAG has a 3.15 times higher effect than G, HAS – 2.66 times higher than that of S, while HAM – only 1.65 times higher than that of M.A significant difference is therefore observed between HAS and HAM, determined by the difference between S and M (M's hyperpolarizing effect is 1.93 times higher than that of S), which is derived from the symmetry difference of the M molecule compared to S, although both substances have the same raw formula and the same type of optical activity. In the case of the acetylated products, it is nevertheless observed that a product lipophilicity is the more efficient the more acetyl groups are situated on the same part of the molecule, thus inducing a more precise polarization of the molecule lipophilic and hydrophilic properties (HAS comparatively with HAM).



The polyphenolic fractions (MF and EF) have as effect the membrane depolarization, which is also correlated with the activity of the $Na^+$–$K^+$ pump, and with the membrane passive permeability, yet the mechanism of the bioelectrical effect of such agents is wholly different from that of the polyolic derivatives (2). In such a case, the membrane depolarization is the consequence of a decreased packing degree of the membranary structure, concomitantly with an increased membranary fluidity and a labilization, which causes an increase of the transmembranary passive ionic flows and also a decrease of the ionic gradients. Nevertheless, this also induces an intensification of the active ionic transport (increase of the $Na^+$–$K^+$–ATP–ase activity) (2), a certain stimulation of the cell metabolism being also observed (7), (8).

The obtained data call for new investigations, aimed at elucidating the mechanisms of the phenomena observed, for possible biomedical utilizations of the products considered for the study (1), (5–8).

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# PROBLEMS OF THE ZOOGEOGRAPHY OF ROMANIA

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La réalisation d'une synthèse zoogéographique régionale nécessite de nombreuses informations des domaines des sciences biologiques (taxonomie phylétique, écologie, paléontologie) et géologiques (paléogéographie générale, paléohydrographie, paléoclimatologie). On considère en general que la Roumanie n'a pas appartenu au réfuge ponto-mediterraneen, ayant été peuplée surtout par des immigrants post-glaciaires. Pourtant la majorité des espèces d'eau douces et hypogées de Roumanie, de même qu'un certain nombre d'espèces terrestres épigées ont un âge préglaciaire dans le pays. Les groupes ("cercles") d'éléments faunistiques terrestres doivent être delimitées surtout d'après le réfuge glaciaire dont ils proviennent. On suggère que les éléments préglaciaires ont survécu surtout dans trois zones du sud de la Roumanie, qui ont été aussi le plus intensivement colonisées par des immigrants post-glaciaires. Une évolution intra-spécifique récente a pu avoir eu lieu dans deux massifs montagneux isolés; quatre zones du nord du pays ont offert les meilleurs conditions de survie auximmigrants d'origine nordique et deux larges vallées ont été des barrières, empêchant ou limitant les dispersions.

## INTRODUCTION

Zoogeography is a synthetical biological science, whose aim is to explain how and when the faunas of various areas originated and evolved. It is promoted mainly by qualified taxonomists, able to combine the results of their own investigations with those of specialists in other animal groups and with what is known about the evolution of the area(s) dealt with and to realise true syntheses.

Inventory, as complete as possible, of the species present in a given area and detailed data about their distribution within and outside this area represent only a first step for the elaboration of a scientific zoogeographical study. A second step is the grouping of the species having identical or similar general ranges, and presumed to have undergone the same dispersal history, in special categories ("circles of faunistic elements").

It is illusory to believe that it will be possible to realise, in the next years and decades, a complete inventory of the fauna of Romania. This is not possible for any European country, for any state of the U.S.A. There are no specialists able to determine the species belonging to numerous families of insects, to several classes and orders of the so-called "worms". Most species of various groups of lower invertebrates have very wide ranges, being irelevant for zoogeography. Zoogeographers must restrict their activity to the animal groups whose species composition, distribution etc. in Romania are sufficiently well known. Some papers have already been published about the zoogeography of some of these groups: fishes, herps, birds, continental snails, orthopterans, caddisflies, some families of beetles and several hypogean groups. In the meantime, the species composition

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and distribution of other groups, especially of fresh water and terrestrial insects, became sufficiently well known for allowing zoogeographycal analyses.

## PREMISES

It has already been mentioned that inventoring of species, maping their distribution, even their grouping in circles of faunistic elements represent only initial steps in modern zoo- and biogeography. Much more information is necessary, pertaining to biological and geological sciences, namely: phyletic taxonomy, ecology, paleontology, paleohydrography, paleoclimatology, palinology.

### PHYLETIC TAXONOMY

It is important to know which are the phyletical affinities of the species inhabiting a certain area, especially of the endemics. As endemics it must be designated all species with a restricted range, not obligatorily a single country: terrestrial species can be endemic to the Carpathians, or to the eastern Carpathians, for ex. *Triturum montandoni*, whose range comprises the Eastern Carpathians, in four countries. Freshwater species can be endemic to the Danube River basin ( its range extends over 11 countries) or to the Tisza River basin (in almost five countries). Determination of phyletical affinities actually means determination of the closest related "sister species". It is obvious that the zoogeographical significance of endemics from certain areas is different if their sister species live in the neighbourhood, or in some remote areas. For example, the sisters of certain fish species endemic to the Danube River basin live in the rivers Nistru, Dnieper or Don (i.e. in the close vecinity), while the sisters of other endemics occur in East Asia or in North America. It is also important to know whether the range of the sister species is allopatric (vicariant) or sympatric (totally or partially) with that of the endemic.

In many cases, especially in those of relict colonies it is often necessary to establish the phyletic affinities below the specific level, at the subspecific or even at the population level. It is possible, by using modern technics, for example electrophoretic ones, or a sequence of nucleotids, to determine the phyletic relationships between conspecific populations, even the age of their divergence. Relict steppic colonies in forrested areas may have originated in different periods of the Pleistocene or Holocene. Similarly, the populations from various tributaries of a large river may have originated, by river captures, each from a tributary of another large river. It will be mentioned in this paper that populations of *Cottus poecilopus* from the head waters of several rivers in the drainage area of the Danube, probably originated: some from tributaries of Oder River, others from tributaries of Vistula or Nistru River (Fig. 1). This supposition can be tested by comparing, using modern technics, specimens from the rivers which may have been subject to captures of their head waters, within the species range.



### ECOLOGY

Historical and ecological zoogeography have been and are still considered opposed and conflicting schools, ignoring and minimizing one another. Actually the distribution of each species and lineage depends on historical and ecological factors as well. One main problem of the Romanian fauna is to establish which of the thermophilous species confined to the south of the country have a preglacial (or at least pre-wurmian) age and which have dispersed recently across the Danube. It is obvious that the dispersal abilities of the various species must be considered when trying to solve the problem.

Numerous species of terrestrial animals, mainly among insects but also among molluscs etc., even vertebrates are restricted to a single, or to a few types of vegetation. The late Pliocene-Holocene evolution of the vegetation in Romania and adjacent areas is well known and it is evident that the range shifting of animal species was the same with that of plant associations. It is therefere necessary to establish, for each animal species in which plant association it lives.

### PALEONTOLOGY

Paleontology furnishes informations concerning: (1) the lineages and species which inhabited Romania in the past and; (2) the former distribution of the species which are presently living in Romania, of their closer relatives and of the lineages to which they belong. The second set of informations is much more helpful for reconstructing the genesis of the recent fauna. The occurrence of Dinosaurians in Transylvania during the Cretaceous, of various tropical families of mammals and fishes during Paleogene times has nothing to do with the recent fauna of the country; it is much more important to know for example that Cyprinidae, the dominant fish family in Romania and in the whole Europe has fossil recording in East- and South Asia since Paleocene times, entered Europe 30 m.y. later and many of its Central European species, also present in Romania, lived in Siberia until late Pliocene times.

The ranges of recent species underwent wide extensions and regressions during the Quaternary climatic oscillations. Valuable contributions on the late Pliocene and Pleistocene micro-mammals (23,24,25), equids (21), snakes (29) give useful informations in this respect. Similar studies on snails, especially on the members of the genus *Alopia*, endemic to the Carpathians are necessary. The most remarkable inland water snail from Romania is *Melanopsis parreyssi*, endemic to the thermal pond "Baile Episcopesti", in county Bihor, the most northern member of a circummediterranean genus, that was widely distributed through Europe during Tertiary times. Fossil members of the genus have been found in the Miocene and Pliocene beds of the county Bihor, in the vicinity of the thermal pound "Baile Episcopesti"; it is necessary to establish their relationships with the recent species.

## PALEOGEOGRAPHY

It is especially useful to know the exact age of the orolift of the Carpathians and the former direct connections between south-eastern Romania (and north-eastern Bulgaria) and Crimea and the Caucasus across the present-day Black Sea; the so-called "Euxinic" or "circum-Pontic" elements may have used these connections.

## PALEOHYDROGRAPHY

Large rivers are uncrossable barriers for numerous species and lineages of terrestrial animals and plants, being on the contrary dispersal routs for numerous groups of freshwater animals.

The dispersal of numerous species and genera is a consequence of the modifications of the riverine net and the following examples deserve mention:

- the euryhialine or strictly freshwater species endemic to the drainage areas of the rivers on the Black and Caspiane seas watershed and belonging to primarly marine lineages are not Pontian (Pliocene) or even Sarmathian (late Miocene) marine relicts in inland waters as formerly believed, but Neo-Euxinic (Wurmian) ones. These species actually have been present in the united brackish water Ponto-Caspian basin during the Pontian Period, but became later extinct from the Black Sea, surviving only in the Caspian Sea, from where they recolonized the Black Sea late in the Neo-Euxine epoch of the Quaternary (16). The Neo-Euxine Sea extended along the Danube only to Lacu-Sarat, near the town Braila. Hence the species present in the Danube only downstream from Lacu-Sarat can alone be considered marine relicts in inland waters; those present also upstreams are autoimmigrants.

- The Danube is, in its present-day shape and size, a young river. The connection between the upper/middle and the Lower Danube in the Iron-Gates area took place late in the Quaternary (5) the upper/middle Danube having been an independent river flowing through the Panonian lake and southwards into the Aegean Sea, probably along the Vardar River valley (1). This explains the occurrence in Vardar (and south from it in Aliakmon River) of six fish and one mollusc species of the Danube fauna which are absent in the other Balkan rivers (1). It will be useful to establish, by using modern technics, the affinities between the populations of several species from the Vardar, from the Danube tributaries (especially from those near the Iron Gates) and from Balkan rivers, east of the Vardar.

- The most significant groups of freshwater animals (fishes, higher crustaceans, unionaceans, mussels, prosobranchiates and others) can disperse from one river basin to another only when a river capture takes place. River captures usually do not affect directly two main rivers, but only one tributary of each of them. A species having colonized a tributary of a large river can only later extend throughout the entire drainage area of this river, if its ecological requirements permit this. Inhabitants of headwaters are often unable to do this and the occurrence of strictly rheophilic species in the upper reaches of several rivers of the same drainage area which are connected only in lowlands can result from distinct river captures. A good example



is furnished by a fish, the sculpin (*Cottus poecilopus*) with a restricted and disjunct distribution in the Danube basin: (1) headwaters of three or four tributaries of the Danube proper in western Slovakia; (2) headwaters of a few western and northern tributaries of Tisza River in eastern Slovakia; (3) headwaters of the upper Tisza River and of its northern and southern tributaries in the Ukraine and Romania (4) headwaters of the eastern most northern tributaries of the lower Danube, Siret and Prut in Romania and the Ukraine and their tributaries. The species is also present in the drainage areas of the rivers Oder, Vistula and Nistru (Dniester) and it seems obvious that it reached the tributaries of the Danube by means of at least four (possibly more than ten) river captures. A comparison, by means of modern methods, of the populations from the various tributaries of the Danube and those of the tributaries of the three rivers, from which they are assumed to have originated can clarify the problem (Fig.l).

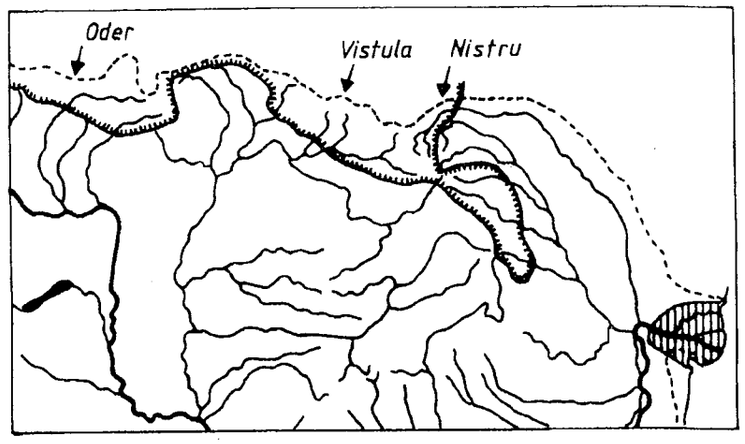


Fig. 1. – Drainage areas of rivers from which *Cottus poecilopus* could colonize various tributaries of the Danube.

Numerous terrestrial species of animals and plants are present both south and north of the Danube in the Iron Gates area. Many of these are assumed not to have been able to cross the river and some authors considered them to have a preglacial age in the Banat (southwestern Romania). However, since the Iron Gates have a more recent, Wurmian age, it can be accepted that these species reached their present range during the RissWurm interglacial period.

## PALEOCLIMATOLOGY

The Carpathians alone have been covered by glaciers; the rest of Romania remained unglaciated, but under climate conditions incompatible with the ecological

requirements of most members of the Pliocene and recent fauna and flora. Certain oases with warmer temperature are however assumed to have existed. Certain species of the terrestrial Pliocene fauna and flora could survive in them. Special investigations are necessary in order to identify these oases and their extension.

### PALINOLOGY

While animal paleontology furnishes only uncomplete informations about the past occurrence and distribution of the members of a limited number of animal groups (especially vertebrates and molluscs), palinology ofers more complete data about the past (mainly late Pliocene, Pleistocene and Holocene) distribution not of single species of plants, but of the whole vegetation. Many species of terrestrial animals (not only of insects, but also of molluscs etc., even of reptiles and mammals) are confined each to a certain type of vegetation. Palinological studies are in an advanced stage in Romania, the evolution of the vegetation since the late Pliocene being rather well known. Zoogeographers can use the results of palinological investigations; but for using them, it is necessary, first of all, to establish which are the animal species characteristic to each type of vegetation.

## DISCUSSION AND CONCLUSIONS

### 1. PROBLEMS OF THE PREGLACIAL ELEMENTS IN FAUNA OF ROMANIA

Starting with (20) and even (17) most biogeographers believed that Central Europe has been depopulated during the Ice Age, the preglacial fauna and flora having been replaced by cold-adapted ones of artic origin. (13, 14) expressed a similar opinion; according to him, the Mediterranean refuge included, in the south-east of Europe, only Greece and the Adriatic Sea watershed.

This wiewpoint concerns only the epigean terrestrial fauna. It is since long accepted (27, 28) that, far from having been depopulated during the Ice Age, the basin of the lower Danube (i.e. mainly Romania) has been the main survival area of the preglacial fish fauna and the main centre from which central, western and partially even northern Europe have been reporulated with fishes. This is proved by the fact that, with the exception of few marine derivatives, all fish species from western and northern Europe are also present in the lower Danube and only some of them south of the Danube drainage area. Also many freshwater invertebrates from Romania (and from the Danube River drainage area in general) are believed to have a preglacial age (1).

A preglacial age is assumed also for the majority of the hypogean invertebrates, at least for those inhabiting caves of the Apuseni Mountains, of southern Banat and of the southern Carpathians, west of the Olt River basin (9).

The problem raises whether preglacial elements survivved also among the epigean terrestrial animals. Endemics, or species shared only with more northern areas (i.e. absent from the Balkan countries south of the Danube) must be considered



first; their absence south of the Danube suggests that these could not have originated from of the Ponto-Mediterranean or Balkan refuge. It is however posible that a few of them or their direct ancestors actually colonized Romania from this refuge, having later become extinct from the refuge, or that the imigrants evolved to distinct species. Only two of the more than eighty plant species endemic to Romania (some even to northern areas of the country) are believed to be preglacial relicts (18). This means, the others have recently evolved to distinct species from postglacial immigrants.

Such a rapid evolution is less probable for animal species, but not totally excluded.

A number of caddis flies, interelated and related also with species from the northern Carpathians in the Ukraine and Poland (2, 3) are endemic to northern Romania. These species, or at least the lineage to which they belong, evidently had a preglacial age in the northern and eastern Carpathians. The same is true for *Triturus montandoni*, a newt, endemic to the eastern Carpathians from Poland to Romania; its absence not only from the Balkan Peninsula, but also from the south-western Carpathians, and the absence of relatives in the same areas are strong arguments in favour of its preglacial age. The same is probable for the numerous terrestrial snails of the genus *Alopia*, each endemic to a certain limestone montane massif from the western, southern and eastern Carpathians. Even the general range of the genus extends only slightly beyond the Carpathians.

A special category is that of the "glacial resistants" more widely distributed European or Euro-Siberian species believed to have survived in central Europe, including Romania, during the Ice Age since the Pliocene. STUGREN (22) lists a few reptiles and amphibians as glacial resistents. To the same categories may belong species from other groups.

### THE DELIMITATION OF VARIOUS CATEGORIES OF "FAUNISTIC ELEMENTS"

One main problem of zoogeography (and phytogeography) of Romania is to delimite the "elements" of the terrestrial epigean fauna (and flora). Most authors, including recent ones delimit these on the basis of their present-day distribution. For example (12) mentions among the butterflies from the Banat: siberian (actually euro-siberian), ponto-oriental, mediterranean, central-european and boreo-alpin species; (10, 11) distinguishes, u.o., species of balkanic, central-asian and pontic origin among the orthopterans of Romania; (15) speak about holarctic, european, mediterranean, palearctic, balkanic, east-mediterranean, west-palearctic, south-east european, north-mediterranean species of chilopods.

According to (19) the bird fauna of Romania comprises 35 species of arctic origin, 44 of siberian origin, some "transpalearctic" ones, besides species belonging to several "types": european, mediterranean, tibetan and chinese.

Few Romanian authors tried to ascribe the terrestrial, epigean species of animals to the "circles of faunistic elements delimited by (13, 14) on the basis of their probable postglacial dispersal from the different glacial refuges in which they

have survived during the Pleistocene glaciations (Fig.2). DE LATTIN, followed the opinion of REINING (17) and (20) that there have been distinct refuges of the arboreal and eremial (steppic) animals, and plants, hence distinct arboreal and eremial circles of elements. While (20) recognizes six refuges for the Eurasian arboreal fauna, (only three of them have contributed to the postglacial repopulation of Europe: the mediterranean, the armeno-persian and east-asian one, DE LATTIN accepts 14 arboreal refuges in Eurasia, three close enough to Romania (Fig. 2) to contribute to its postglacial colonisation: the mediterranean, the caspian (better to design

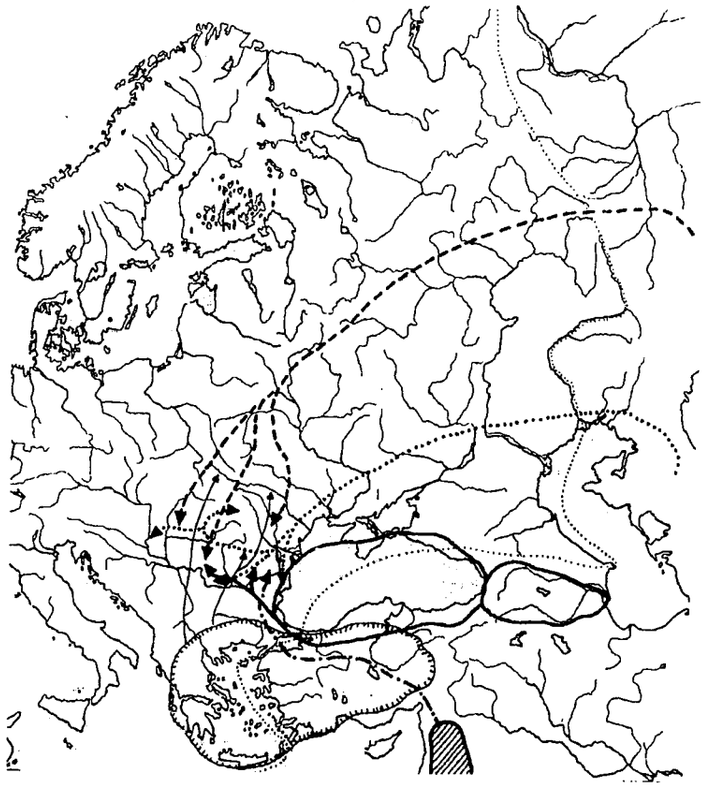

Fig. 2 – Barred line – ponto-mediterranean subcentre; continuous line – caucasian centre; oblique lines – syrian centre; interrupted line – dispersal route of the ussuric elements: dotted line – dispersal route of the eremial turanic element.

it as "caucasian") and the syrian ones. He also accepts three subcentres of the mediterranean centre: the ponto-mediterranean, (the Balkan Peninsula and western areas of Anatolia), the adriato-mediterranean or italian and the atlanto-mediterranean one, (the Iberian Peninsula and northwestern Africa). Some species have survived in all three subcentres having colonized central Europe from three directions: these are holomediterranean elements. More numerous are the ponto, adriato and atlanto-mediterranean elements (Fig. 2).



The manchurian centre, that comprised the north of the continental eastern Asia, consisted of three subcentres, too. One of these the ussurian subcentre of the manchurian played an important role in the postglacial colonisation of Siberia and Europe (Fig. 2):

DE LATTIN accepts nine refuges/dispersal centres of the eremial fauna of Eurasia and northern Africa: the northern ones for steppic animals and plants, the southern for desertic organism. Most steppic animals from Romania and Hungary (and in general from eastern and central Europe) originated from the turanic or aralo-caspian centre (Fig.2). This extended in the arid areas east of the Caspian and south of the Aral seas, probably also west of the Caspian Sea. Most steppic mammals, birds, reptiles, insects etc. now living in the Ukraine, Moldova, Romania and Hungary originated from this centre; a few birds from Romania are believed to have originated from the tibetane and mongolian centres.

Finally, the inhabitants of arctic and highalpine tundras and similar biotops are designed as arctoalpine elements. They are assumed to have originated from extreme northern Eurasia, that enjoyed during the late Pliocene of a cold climate. During the Ice Age, the arctic fauna and flora extended over most of Europe, south of the ice cup and retired in post-glacial times both in the north of the continent and in the high mountains. Most arcto-alpine elements have presently a disjunct range and are designed as arcto-alpine relicts. While the arboreal and eremial elements are presently in an expansive phase (and were in a regressive phase during the Ice Age), the arcto-alpine elements are in a regressive phase and have been in an expansive one during the Ice Age.

The glacial refuges have enjoyed during the Ice Age a colder and more humid climate than now and were populated by different faunas. The circum-mediterranean areas, corresponding to the mediterranean refuge (Fig. 2), had a humid climate similar with the recent one of central Europe. They were covered by deciduous forests and populated by a fauna identical or almost identical to the forests and fauna now in central Europe. The postglacial warming was accompanied by aridization; the former mediterranean refuge, especially its southern half, is now populated mainly by an eremial, semidesertic fauna and flora originary from the afro-eremial centre. The mediterranean elements represent now only a minority in the mediterranean centre, some are restricted in the mountains of northern Africa, Spain or Italy, others became even totally extinct from the refuge, being present only in central Europe. Species which are, historically, mediterrannean elements can be designed on the base of their present range, as european or central european.

The above mentioned facts clearly demonstrate that the delimitation of faunistic elements after the present range of the species is not justified. The historical factor must be considered first; present day ranges, as well as ecological requirements of the various species ascribed to the same circle of faunistic elements can be used in subdiving these circles in groups of species.

The ussuric elements have a wide usually continuous eurosiberian range; most or all of them still surviving in the ussuric centre which, unlike the mediterranean one, retained to a great measure its former climatic conditions. All species inhabiting coniferous forests, in Siberia, central Europe and the mountains

of Romania (even in some mountains of southern Europe) are ussuric elements. But many ussuric elements also live in deciduous forests and in humid meadows; the ranges of some of them extend to northern Spain, central Italy, the Balkan Peninsula as far as southern Bulgaria (examples and maps in DE LATTIN, 1967).

Groups (or subcircles) of ussuric elements can be delimited mainly on the basis of their thermophily. The less thermophilous ones are confined to coniferous forests. These were the first to extend westwards from the ussuric centre, in Siberia and Europe. When Europe had a colder climate, these elements had a wide and continuous range in the continent; as a consequence of the subsequent warming of the climate, they retired northwards and in the mountains. Their range became disjunct: they are boreo-montane species or elements, which must not be confounded with the arcto-alpine ones. The later ones are oreo-tundral elements which inhabit tundras, north of the forest belt and high mountains above the forests, while the boreo-montane species are inhabitants of the coniferous forests both in the lowlands of northern Europe and the hills and mountains of Central Europe, including the Alps, the Carpathians etc.

The ussuric elements represent a high percentage of the Romanian fauna. Even in the southern province, the Banat, that also enjoys a warmer climate, the ussuric or siberian elements are dominant (i.e. among the butterflies, they represent 65% of the fauna - 12).

The mediterranean elements deserve special mention. As clearly demonstrated by REINIG, DE LATTIN and others, these elements extended from the mediterranean refuge to Europe, mainly to central Europe in a wide sense, some even to western Siberia, but only few of them to the north of the continent. Most species designed as european, south european, euromediterranean, sub-mediterranean, etc. are actually mediterranean elements. Some species having an european and especially a central european range are glacial resistents survivors of the preglacial fauna. In the absence of paleontological documents, it is however difficult to distinguish between glacial resistents and mediterranean postglacial immigrants.

On the basis of the northern extension of the mediterranean species, of their temperature requirements and of their survival or extinction from the refuge, several "subcircles" of mediterranean elements can be delimited. DE LATTIN makes distinction between mediterranean elements in a stationary phase which dispersed only slightly beyond the limits of the refuge, and in an expansive phase, which extended farther north.

Many mediterranean elements present in Romania are holo-, adriato-, apparently none atlanto-mediterranean, but many possibly the majority, are ponto-mediterranean. In the light of DE LATTIN's views, it is not justified to make distinction between ponto-mediterranean and balkan elements. However, future studies may give the possibilty to distinguish several groups of ponto-mediterranean elements. The ponto-mediterranean centre had a wide extension, some of the species later to extend northwards to Romania may have been initially confined to a restricted area of the refuge. It is worth mentioning that botanists distinguish between illirian (more western) and moesian species. Such distinction could be possible also for animals. On the other hand, the illirian elements may have extended not from the western



part of the ponto-mediterranean subcentre as delimited by DE LATTEIN, but from the eastern area of the adriato-mediterranean subcentre (i.e. from the dalmatian coast of the Adriatic sea).

It has been mentioned that a number of Romanian or Carpathian endemics are probably preglacial survivors. Certain areas are believed to have enjoyed a comparatively mild climate during the Ice age, permitting the survival of *Triturus montandoni* (in the eastern Carpathians), of several *Alopia* species (different limestone massifs in the Carpathians), and of a few plants (*Hepatica transylvanica, Syringa josikea*). These small areas in which *T. montadoni*, the numerous endemic species of *Alopia* and possibly other preglacial elements survived, can be considered as parts of the mediterranean refuge or as "fragments" of this refuge. It is obvious that the glacial refuges did not offer identical life condition on their entire extensions; the conditions were more favorable in the southern parts of all refugies and became gradually worse in their northern parts; also the number of species was higher in the south. The localities of Romania in which preglacial species survived belonged to the extreme north of the pontomediterranean refuge.

It has been mentioned that the drainage area of the lower Danube has been the main refuge of the Pliocene fauna of fishes and probably of other aquatic groups. It has played the same role, for aquatic animals as the ponto-mediterranean refuge for the terrestrial ones.

The caucasian elements are less numerous in Romania than the ponto-mediterranean ones. A number of reptiles, molluscs, butterflies and bugs are caucasian elements. These are mainly inhabitants of meso-xerophilic forests, characterized by a warmer and drier climate than the typical central-european forests inhabited mainly by mediterranean elements. The caucasian elements are, to a certain extent, ecologically intermediate between the arboreal and the eremial elements. Most invertebrates characteristic to meso-xerophilic forests may be caucasian elements.

Most species living in alpine habitats above the forrests are oreo-tundral elements. But not all are also present in the arctic tundra, many are confined to the mountains of Europe. These will probably be ascribed in the future to distinct circles of faunistic elements.

The majority of the inhabitants of arid steppes in eastern, southern and western Romania as well are turanic elements.

Only quite few animals from Romania have been identified as syrian elements.

### 3. RELICT SPECIES AND COLONIES

The meaning of "relict" in biogeography refers exclusively to the manner in which a species or colony has reached its range, without considering the degree of archaism or the age of the species. As clariy pointed out by (4): a species is a relict in a region or locality if its occurrence there can be explained only by accepting that it or its direct ancestor reached there when the geographical and/or climatic conditions were different from the present day ones. The same species can be relict

in one or several localities and immigrant in others. The term relict implies range disjunction(s), either of the conspecific populations or of the species of a lineage: the arcto-alpine species live in the high north and in high mountains, being absent from the intermediary areas; the European species having closest relatives in East Asia or North America and having no living relatives in Siberia are preglacial relicts.

Many European species and genera are present also in East Asia and absent from Siberia (the genera *Bombina* among frogs, *Misgurnus*, *Rhodeus sericeus*, *Cyprinus carpio* among fishes. These are preglacial relicts in the continent; the same can be asserted for the monotypic fish genus *Romanichthys*, endemic to a small area of Romania and related to some American genera.

The biogeography of these widely disjunct relict genera concern the fauna of the entire Europe. It is worth mentioning that some of these genera and species-groups are confined to Romania and neighbouring areas or countries: the above-mentioned *Romanichthys*, the snail genus *Holandriana* (= *Amphimelania*) and others·

More interesting for Romania are the disjunctions determined by the alternative climatic oscillations during the Pleistocene and Holocene. The four main glaciations and the numerous climatic oscillations determined southwards shiftings of the whole biotas during the glacial periods and northwards shiftings during the interglacials. Often a biota retreated when climatic conditions became unfavorable, but few specimens could survive in localities where the conditions were less unfavorable. These relicts built colonies.

The following categories of relicts are present in Romania:

– glacial or arcto-alpine relicts in high mountains, some also at lower altitudes, for example in bogs. Most arcto-alpine relicts are believed to have a wurmian age; during the wurmian glaciations they had a continual range at lower latitudes south of the northern ice-cup and at lower altitudes under the alpine ice-cup. It is, however, theoretically possible that some have a Riss or Mindel age;

– boreo-montane relicts; typical inhabitants of coniferous forests, which migrated from the ussuric refuge westwards at the beginning of the postglacial period, when the climate was colder than now; as the climate became warmer, the coniferous woods and their animal inhabitants retired to the northern and the montane taiga (but not to the arctic or high alpine tundra);

– thermophilous relicts; in certain post-glacial periods, and during the interglacials, the climate was warmer than now, the mediterranean elements extended farther north than presently. As the climate became cooler, these elements retired southwards, but left some relicts colonies in localities offering adequate conditions (for example limestone mountains). It is worth mentioning that the species and subspecies of the snail genus *Alopia* are confined to limestone hills or mountains. Some of them may be relicts from the warmer periods of the postglacial, even from aninterglacial period.

– relicts colonies of steppic animals in woodlands in central Europe: in an arid phase of the Postglacial period the eremial flora and fauna extended farther west and northwards than now, they retired when the climate became again humid, but left steppic colonies in some adequate sites, for example *Vipera ursini* and *Spalax istricus* in a few localities from central – northern Transylvania (Câmpia Ardealului).



Deforestation, extension of cultivated fields in former woods and steppes during quite recent historical times determined further range disjunctions of both arboreal and eremial (steppic) biotas: their isolated colonies can be designed as "cultural relics".

## 4. ZOOGEOGRAPHICAL PROBLEMS OF CERTAIN AREAS OF ROMANIA

Southern Banat, western Oltenia and Dobrogea (including the Danube Delta) are the areas comprising the highest percentages both of termophilous preglacial elements and of postglacial immigrants from the south (Fig. 3). Special investigations are necessary in order to distinguish between both categories and

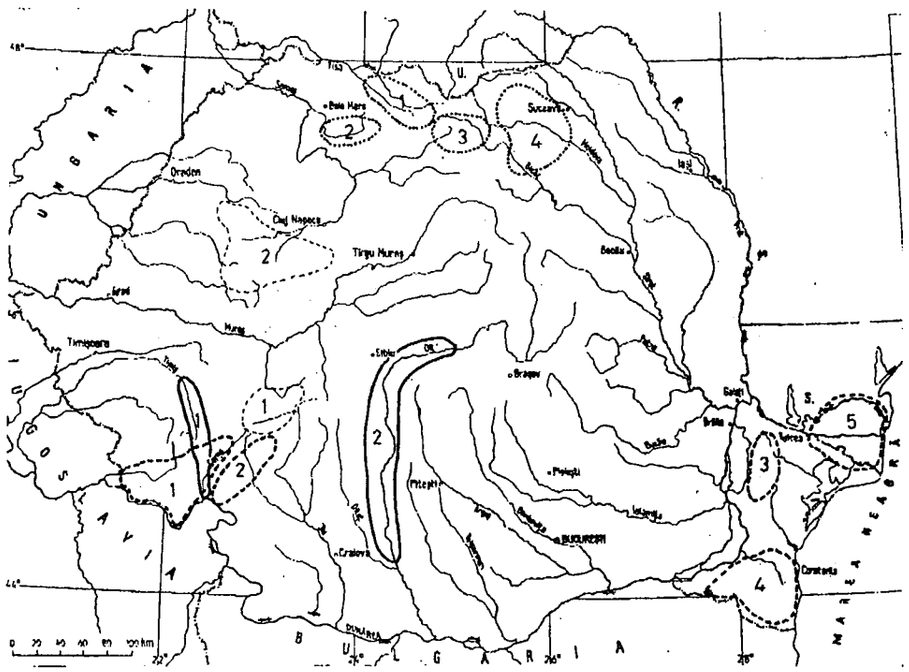


Fig. 3. – Areas of Romania deserving special zoogeographical interest

– – – – areas including the highest number of thermophilic species (preglacial survivors and postglacial immigrants): 1-southern Banat; 2-western Oltenia; 3 and 4-areas of Dobrogea; 5-Danube Delta;

. . . . . northern areas, assumed to include a higher number of glacial relics: 1 - Maramures; 2 - Valley of Lapus River; 3-Rodna Mountain; 4- forrested areas in northern Moldavia;

– – – – isolated montane massifs in which the process of speciation could be more intensive: 1-Retezat Mountains; 2-Apuseni Mountains;

_____ areas that represented migration ways and at the same time barriers; 1 – the Timis-Cerna passage way 2 – the Olt River valley.

especially between ponto-mediterranean (balkanic), caucasian and syrian elements, their distribution patterns in Romania and their ecological requirements.

Several northern areas from Romania - the province Maramures, the Rodna mountains, the valley of Lapus River and the forests of northern Moldavia, are the areas where glacial relicts are assumed to be more numerous. A relatively high numer of species have been described from the terrestrial habitats of the Danube Delta and are often listed as endemic. But the Delta is quite young (a few thousands of years) for having permitted speciation phenomena. It is necessary to investigate the areas where these species may be present and to test their dispersal abilities (many of them may be imigrants from Crimea and the Caucasus).

The Retezat mountains and the Western Carpathians (Apuseni mountains) are among the most isolated mountain massifs in Romania and several phenomena of intraspecific isolation may have taken place.

The wide depression between the mountains of southern Banat and the Tarcu-Retezat massif, that is drained by Timis and Cerna rivers "culoarul Timis-Cerna" is assumed to have been at the same time a migration route for some southern (Balkanic) animals and a barrier for other species.

The valley of the Olt River is another barrier for the terrestrial fauna.

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