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QUELQUES REMARQUES SUR LA TAXONOMIE DU GENRE *MESONISCUS* CARL

PAR

V. GH. RADU

In this paper, the author shows on the morphological-comparative, statistical and zoogeographical basis the taxonomical value of second pleopodal endopodit of the male of *Mesoniscus*, an organ which has not received so far a proper attention.

La dernière révision taxonomique, due à H. E. Gruner et I. Tăbăcaru [1], conclut, sur des bases bien fondées, que le genre *Mesoniscus* possède seulement deux espèces, *Mesoniscus alpicola* Heller 1858 et *M. grangier* Frivaldsky 1865. A cette occasion, nous croyons juste de rappeler que nous avons publié, nous aussi, une révision critique sur le *Mesoniscus alpicolus vulgaris* Chappuis (1944) [2] remarquant l'erreur de P. Chappuis (1944) de créer trois sous-espèces de *Mesoniscus alpicola*, toutes dans les grottes des M^{ts} Apuseni, appuyé sur la constitution des poils du propodite des péréiopodes VI et VII, qu'il avait mal observée et faussement interprétée. Gruner et Tăbăcaru ont omis de mentionner notre contribution à la taxonomie du genre *Mesoniscus*. Aussi, ils ont sous-estimé l'affirmation de Verhoeff [4] sur l'existence d'un segment terminal de l'endopodite, privé d'épines sur le bord interne, sur le matériel de Hallstatt, Kirchberg et Mariazell. Ils n'en font pas même mention dans leur travail. Nous avons retrouvé cette particularité sur le matériel collecté dans les Carpates Orientales et c'est justement ce fait qui nous a incité de faire cette communication.

MATÉRIEL ET MÉTHODE

Une grande quantité de matériel a été mise à notre disposition par le Dr C. Pleşa, de l'Institut de Spéologie de Cluj-Napoca, qu'il a récolté dans les nombreuses grottes des M^{ts} Apuseni. Nous lui exprimons nos remerciements. Nous avons collecté nous-même un riche matériel dans les grottes des M^{ts} Apuseni et dans les Carpates Orientales, de Dorna-Rarău, jusqu'à Buzău—Vrancea, pas dans des grottes, mais sous des amas ou de gros blocs de pierres. Une belle collection de *Mesoniscus* a été temporairement mise à notre disposition par le Musée d'Histoire Naturelle de Vienne. Nous en remercions vivement M le Dr Kritscher, le directeur du Muséum.

Dans la présente étude nous envisagerons exclusivement la constitution de l'article distal de l'endopodite du pléopode 2 mâle. Notons que les affirmations selon lesquelles l'endopodite respectif serait constitué par 3 articles sont assez faiblement fondées et par suite nous considérons que cet appendice ne possède que deux articles. Notre but est de chercher à préciser si la portion terminale de l'endopodite présente une conformation permanente, non stadiale, caractéristique pour les mâles adultes, et de constater l'étendue et la fréquence des conformations différentielles dans les diverses populations régionales de *Mesoniscus*.

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Pour le développement stadial, nous nous sommes heurtés à l'inconvénient qu'à la fixation du matériel dans l'alcool, les individus ne restaient pas tous dans le même état de contraction ou de distension et il nous a été souvent difficile d'établir exactement leur longueur réelle. Pour un repère plus sûr, nous avons pris en considération la longueur de l'article terminal de l'endopodite qui ne subit pas des modifications pendant la fixation. En même temps nous n'avons pas sous-estimé l'importance de la corrélation entre le développement des dimensions de cet article et les dimensions (longueur) du corps des individus. L'augmentation du nombre des épines de l'endopodite nous a servi aussi comme critère valable pour estimer le développement stadial, le degré de maturité des individus.

RÉSULTATS

Dans la figure 1 on voit que chez les *Mesoniscus* des M[ts] Apuseni (fig. 1, Aa, Ab, Ac) l'endopodite est pourvu, sur son bord interne, d'une rangée d'épines qui commence à quelque distance au-delà de l'éperon et se prolonge *sans discontinuité* jusqu'au bout extrême de l'article. Sur la face dorsale de cette extrémité il existe aussi quelques épines, en nombre variable.

Chez *Mesoniscus* des Carpates Orientales (fig. 1, B), à la partie épineuse de l'endopodite on peut reconnaître aisément deux portions, une proximale, pourvue d'une rangée d'épines sur son bord interne, et une distale, où cette rangée d'épines fait défaut. Le rapport entre les dimensions de ces deux portions est variable, compris entre 0,4—0,6 pour la partie distale.

Pour les populations des M[ts] Alpes, l'endopodite en cause a, du point de vue de la répartition des épines, la même constitution que celle des Carpates Orientales (fig. 1, C), les deux portions étant en rapport de 0,4—0,55.

Il y a lieu de remarquer aussi deux exceptions. A Sabassa (dans les Carpates Orientales), nous avons trouvé un mâle dont la rangée d'épines de l'endopodite 2 s'étendait sans discontinuité jusqu'au bout. Aussi, à Lurhöhle (dans les Alpes Autrichiennes), la rangée d'épines était complète, mais cette fois-ci, chez tous les mâles que nous avons eu à notre disposition (4 exemplaires) de cet endroit, ce qui doit avoir une certaine signification, d'autant plus qu'ici il y a aussi des détails de structure bien particuliers (fig. 1, D) sur lesquels nous reviendrons dans une future communication.

En ce qui concerne la constance et la permanence, c'est-à-dire le caractère non transitoire de ces deux types de structure du gonopode dans l'ontogenèse chez *Mesoniscus*, la figure 2 nous en fournit la preuve. Tout d'abord, la partie supérieure de chacun des trois graphiques, A, B et C de cette figure montre que dans toutes les trois populations qu'ils représentent, l'endopodite s'accroît progressivement par rapport à l'accroissement en longueur du corps de l'individu.

La partie inférieure des mêmes graphiques représente, chez un grand nombre d'individus, les rapports entre le nombre d'épines existantes sur l'endopodite et la longueur de celui-ci dans différents moments successifs de l'ontogenèse. Dans le graphique A (M[ts] Apuseni), il est à remarquer que les épines de l'endopodite commencent à apparaître au moment où l'article distal de celui-ci a atteint les dimensions de 0,35, 0,4 ou même 0,5 mm de longueur. Avant ce stade, l'endopodite en est totalement

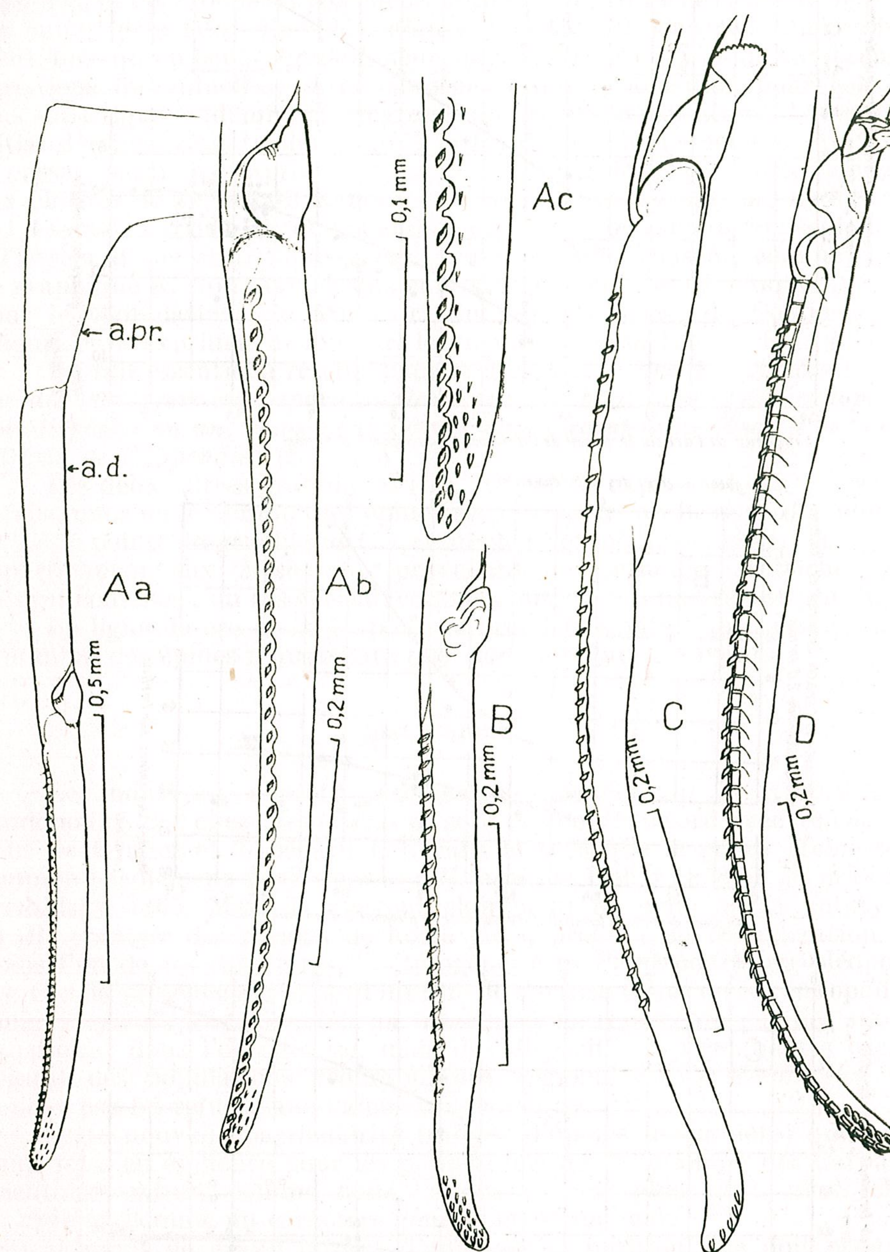

Fig. 1. — L'endopodite du pléopode 2 ♂ chez les espèces de *Mesoniscus*. Aa, Ab, Ac, chez *Mesoniscus graniger* des M[ts] Apuseni. As : l'endopodite vu en entier ; *a.pr.*, article proximal ; a.d., article distal. Ab : la partie épineuse de l'article distal, plus grossie ; Ac : l'extrémité de l'article distal encore plus grossie. B, chez *M. graniger* des Carpates Orientales, la partie épineuse de l'article distal. C, chez *M. alpicola* des Alpes Autrichiennes, la partie épineuse de l'article distal. D, chez *M. alpicola* de Lurhöhle (Alpes Autrichiennes) : la partie épineuse de l'article distal.

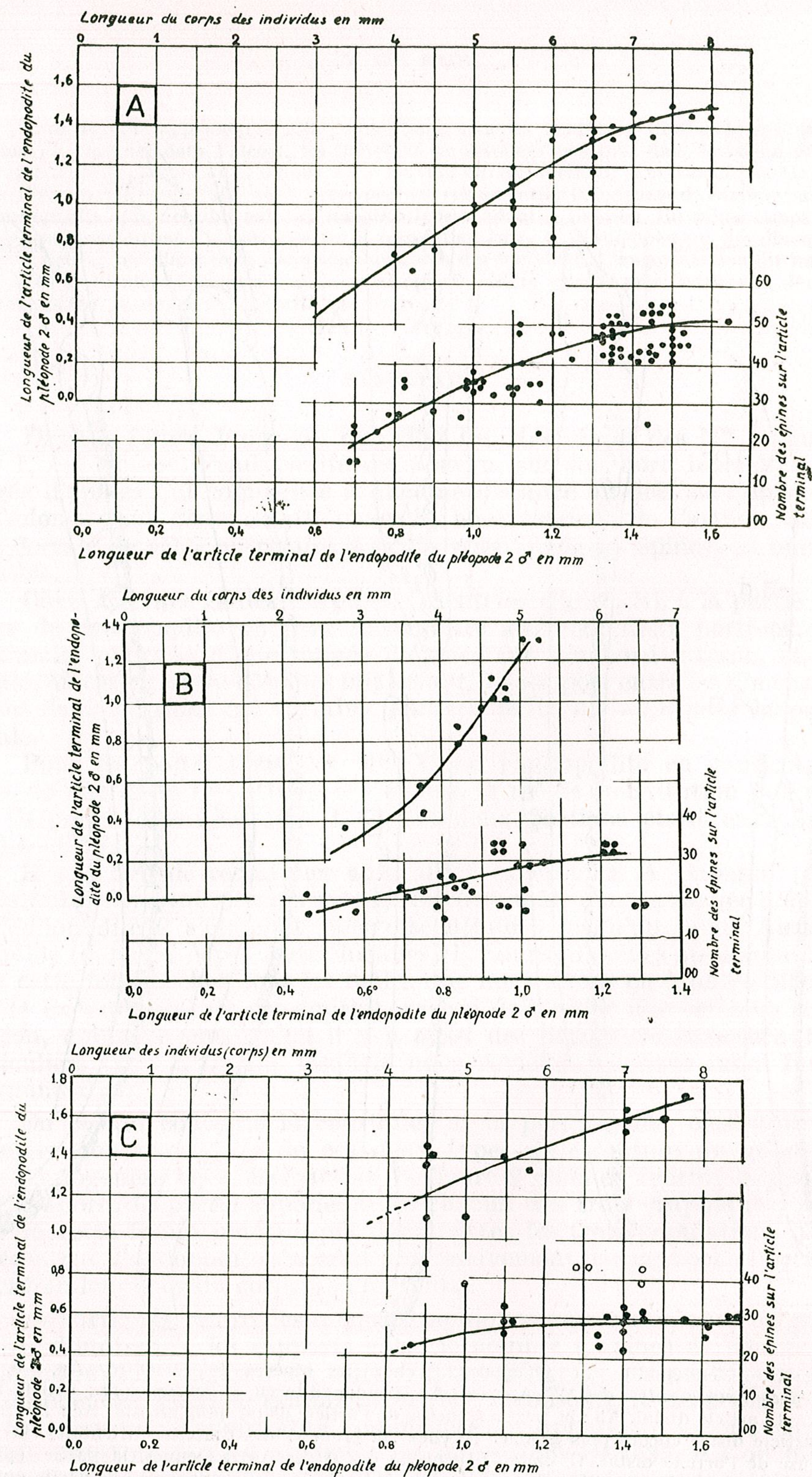

Fig. 2. — Trois graphiques, montrant dans leur partie supérieure, le rapport entre la longueur de l'endopodite des pléopodes. 2 ♂ et la longueur du corps et, dans leur partie inférieure, le rapport entre le nombre d'épines du bord interne de l'article distal et la longueur de cet article. A. Chez *M. graniger* des M[ts] Apuseni. B. Chez la même espèce des Carpates Orientales. C. Chez *M. alpicola* des Alpes Autrichiennes.

dépourvu et l'éperon n'est pas encore formé. A partir de ce stade, le nombre des épines déjà haut d'emblée, d'au moins 15—20, s'accroît progressivement, bien qu'un peu irrégulièrement, dans le sens qu'il y a de nombreuses variations individuelles, entre certaines limites, sans que, pour cela, le sens statistique évidemment progressif du processus soit altéré. Le nombre initial d'épines peut tripler jusqu'à la fin du développement, ce qui peut se passer, paraît-il, d'après le graphique A, en trois étapes, correspondant aux chiffres de 30, 45, 60 épines en moyenne et aux longueurs de 0,8, 1,1 et 1,4 mm de l'article distal de l'endopodite. Ceci correspond non seulement à l'aspect d'une statistique globale pour les M[ts] Apuseni, comme celle du graphique A. Nous avons construit dans ce sens des graphiques séparés pour les populations de Vadu-Crișului, de Corbasca, de Coliboaia, de Măgura et ils exprimaient toujours les mêmes sens que le graphique général.

Un fait essentiel à retenir pour le *Mesoniscus* des M[ts] Apuseni c'est que *dès leur première apparition, les épines du bord interne de l'endopodite sont disposées en une rangée qui s'étend sans discontinuité jusqu'à la pointe extrême de l'appendice* (fig. 1. A).

Les deux autres graphiques (fig. 2, B et C) expriment des processus pareils, mais ici le nombre des épines reste toujours au-dessous du nombre 40. Les 5 points du graphique C qui touchent et même dépassent ce chiffre appartiennent aux *Mesoniscus* provenant de Lurhöhle (Autriche), qui doivent constituer un cas spécial, comme nous l'avons mentionné plus haut.

La ligne de ces deux graphiques prend très vite l'horizontale, donc le nombre des épines n'augmente plus dans les stades suivants.

DISCUSSIONS

Les nouveaux aspects taxonomiques concernant la structure de l'endopodite 2 ♂ chez *Mesoniscus* ne sont pas en désaccord avec les conclusions de Gruner et Tăbăcaru [1], dans le sens que le genre *Mesoniscus* comprend seulement deux espèces : *M. alpicola* Heller 1858 et *M. graniger* Frivaldsky 1865. Mais ils mettent clairement en évidence la polytypie du *M. graniger* des régions de notre pays, prenant en considération au moins l'un de ses caractères, l'hétérogénéité de l'endopodite du pléopode 2 ♂. Car, la présence sur le bord interne de l'article distal de cet endopodite d'une *rangée d'épines complète ou incomplète* ne représente pas des stades transitoires dans l'ontogenèse, mais des dispositions permanentes caractérisant des populations géographiques régionales du *Mesoniscus*. On ne peut pas lui refuser une valeur taxonomique.

Cette nouvelle particularité (rangée d'épines incomplète) que nous avons mise en évidence pour les populations de *M. graniger* des Carpates Orientales existe, comme nous l'avons montré plus haut, aussi chez *M. apicola*, comme un caractère général et permanent.

Il serait de grand intérêt d'analyser la position que doit occuper ce caractère dans la hiérarchie taxonomique des deux espèces de *Mesoniscus*. Momentanément nous nous limitons à opiner qu'on ne pourrait lui attribuer une signification phylogénétique, mais tout simplement un caractère de convergence et nous considérons que cette interprétation n'implique pas du tout la diminution de sa valeur taxonomique, étant donné sa généralité et sa permanence.

Dans nos recherches sur *Mesoniscus* nous avons observé aussi certaines particularités structurales inédites qu'il n'y a pas lieu de décrire ici. Mais, d'après toutes nos observations, nous pensons que l'étude du genre *Mesoniscus* doit être reprise et approfondie de tous les points de vue : morphologique (inclusivement caryologique), écologique, éthologique, zoogéographique, etc. et bien entendu populationnel et stadial aussi. Momentanément, nous croyons qu'il serait suffisamment justifié d'encadrer provisoirement les populations hypogées de *Mesoniscus* des Carpates Orientales au niveau de sous-espèce, pour laquelle nous proposons la dénomination de *M. graniger moldavicus*. (Le nom *moldavicus* dérive du nom Moldova, province roumaine).

CONCLUSIONS

On signale, chez certaines populations du *Mesoniscus graniger* Frivaldsky 1865, la découverte d'un nouveau caractère taxonomique, la discontinuité de la rangée d'épines sur le bord interne de l'article terminal de l'endopodite pléopodal 2 du mâle. Ce caractère a été trouvé seulement chez les populations des Carpates Orientales, pour lesquelles l'auteur propose la création d'un nouveau taxon, la sous-espèce *M. graniger moldavicus* n.s.sp.

Ce caractère existe aussi en général chez les populations du *M. alpicola* Heller 1858, excepté celle de Lurhöhle, mais il a été ignoré ou minimisé par les auteurs.

Basé aussi sur d'autres observations encore inédites, l'auteur est d'avis que l'étude systématique, d'après les exigences modernes, des espèces du genre *Mesoniscus*, est à reprendre.

Localités de provenance du matériel de Mesoniscus étudié

Monts Apuseni (Roumanie). Grottes : Măgura-Sighiştel ; Drăcoaia-Sighiştel ; Coliboaia-Sighiştel ; Corbasca-Sighiştel ; Igritza ; Fatza-Pietrei ; Fînatze ; Călătza ; Vadu-Crişului ; Izbîndiş ; Huda lui Papară.

Carpates Orientales (Roumanie). Sous des amas de pierres ou sous des blocs de pierre : Chiril — Suceava ; Crucea — Suceava ; Sabasa — Neamtz ; Barnar — Bacău ; Slănic — Bacău ; Soveja — Vrancea ; Ciucaş — Buzău.

Alpes Autrichiennes : Grimming-Emstal ; Kaisergebirge-Tirol ; Kasberg-Gipfel ; Oischer N.Ö. ; Grossen-Burchstein ; Graben-Mitterberg ; Mitteralm bei Aflenz ; Kalbling bei Admond ; Lurhöhle.

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HETEROTANAIS LONGIDACTYLUS N. SP. AND *SYNAPSEUDES MEDITERRANEUS* N.SP., TANAIDACEA NEW FOR THE EASTERN MEDITERRANEAN FAUNA

BY

MIHAI BĂCESCU

The examination of a Peracarida material dredged by Dr. G. I. Müller from the Libyan waters on the occasion of a joint study of the fishering reserves of this part of the Mediterranean Sea allowed the author to find, among others, two new species of Tanaidacea described below, as well as *Parapseudes grubei*, to which he gives some morphological completions.

1. **Heterotanais longidactylus** n.sp.
(fig. 1)

Male. Segment 3 of the basis of antennule four times as long as wide, flagellum 6-segmented; its proximal segment bearing 2 tufts of 3 aesthetascae, each of which one appearing as inserted on the last segment of basis. A_2 5-segmented with minute flagellum unisegmented (fig. 1 A).

Carapace very narrowed in the anterior half, to make room for the huge carpus of cheliped (fig. 1 *B*); eyes free, triangular in front view, rounded in lateral view, with large ommatidia, black-brown pigmented; between them rostrotriangular carapace prominent.

Cheliped huge (fig. 1 *A*), with an enormous dactylus nearly twice as long as propodus; the latter with $\pm$ triangular appearance in lateral view, due to a huge apophysis in which its proximal margin projects. Apophysis terminating in a chitinous tooth, followed by another 2 — 3 finer denticles and 3 long, simple setae on its anterior protuberance. Sensitive comb of propodite, transversally situated as against propodus axis, and consisting of 15 — 16 hairs. Besides the genital plate (fig. 1 *D* and *E*) with the two typical hemispheres characteristic of the Dikonophora order, also a strong ventral projection appears among the anterior pleopods (*a*, fig. 1 *E*).

Anterior peraeopods fine (fig. 1 *H* and *I*), the posterior ones with basipodite twice as thick and shorter (fig. 1 *J* and *K*). In both adult ♂♂ examined, uropods show a 5-segmented endopodite and a 1-segmented exopodite (fig. 1 *C*).

Female. Carapace only slightly narrowed at the rostral obtuse triangular frons. A_1 3-segmented (fig. 1 *F*) with a minute flagellary segment; A_2 with 3 short basal segments and 2 long segments at the flagellum. Eyes as well developed as in ♂.

Cheliped small, with short fingers of about the same length (fig. 1 *G*) and a structure common in Leptocheliidae. Peraeopods and uropods as in ♂. Size ♂ = 2.6 mm (with A_1 and uropods = 3.7 mm); another ♂ = = 2.2 mm (3.2 mm respectively).

Material: Libya, St. 105: 1 ♂, 15 ♀♀ (marsupiphore, ± transparent, just after moulting, and 9 juv.; dredge St. 98 Libya, 70 m; coralligenous sand with *Palmophyllum*; date 30.09.1975; associated with 3 *Pleurocope dasyura* Walker and 2 *Caecostenethrioides ischitanum* Fredj & Schiecke. St. 169, 1 ♂ and 8 ♀♀ July 8, 1976.

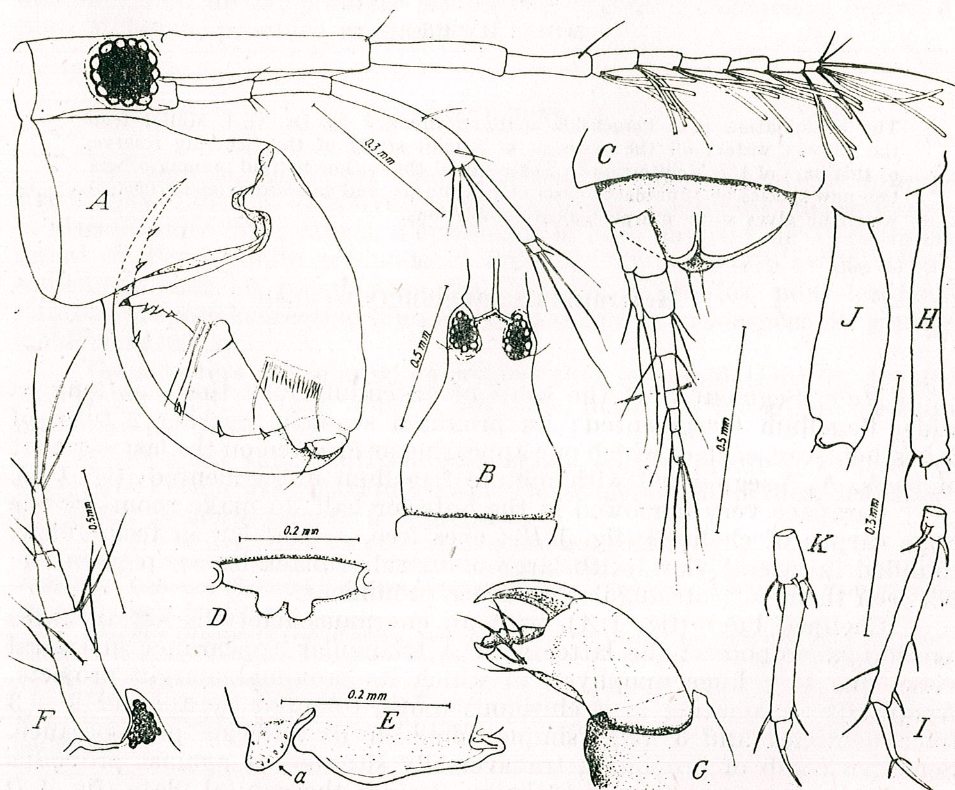

Fig. 1. — *He'erotanais longidactylus* n.sp. *A*, internal view of a male cheliped (♂ St.169); *B*, carapace seen from above; *C*, pleotelson and left uropod; *D*, cross section through the last peraeonite allowing to see the sexual tubercules on the respective plate; *E*, the same in profile; *a*, tubercle of pleonite II; *F*, ♀ antennule; *G*, ♀ chela; *H*, coxa peraeopod II in comparison with the last one (*J*); *I*, peraeopod II; *K*, peraeopod VII.

Holotype ♂. No. 370 in the collection of crustaceans of the "Gr. Antipa" Museum. Allotype ♀, ditto, no. 370 *a* and 1 ♂ + 15 ♀♀. Paratypes, ditto no. 371.

Remarks. Even through the cheliped does not have the posterior, aberrant projection of *Heterotanais oerstedi* and though the apophysis of its carpopodite is more developed than in *H. longitactylus*, the cheliped of the latter species is it least as characteristic of the new species as of the former.

No matter the genus suggested within the Leptocheliidae family to which we would refer it, the Libyan species distinctly differs from all the species considered in *Heterotanais* (*H. anomalus H. algiricus*, *H. magnus*, etc.) by the enormous width of the propodus of cheliped in ♂ and by the great length of the dactylus of the latter, curved in a semicircle. We did not ascribe the species to the genus *Pseudoleptochelia*, recently erected by Lang, because we did not find an adequate morphological justification.

2. Synapseudes mediterraneus n.sp.

(fig. 2)

Description ♀. Body smooth, slightly shining, with similar peraeonites (fig. 2 *A*), bearing four strong setae each — two laterodorsal, two lateral. Frons slightly bilobated with sinuous edge; eyes black. Pleon consisting of 2 well-delimited segments, *both with lateral projections terminating in spines*. Only 4 setae on the tergal face, as well as on peraeonites. Pleotelson large, triangular, with an acute apex in dorsal view (fig. 2 *D*), but like a creast when viewed laterally (fig. 2 *C*, arrow). It shows two small dorsolateral prominences, each bearing 1-2 setae and a small tubercle with 4 strong hairs immediately above the apical crest. The supraanal crest has 3 more individualized denticles, dorsally and posteriorly directed. Antennules (fig. 2 *E*) with stumpy, massive segments; the proximal one, irregularly serrated on its inner margin, is shorter than the combined following ones, being hardly longer than wide; bisegmented flagella. Antennae (fig. 2 *F*) with uniarticulated flagellum, bearing 3 particularly strong setae. Labrum with a strong conical projection; mandible (fig. 2 *G*) with palp having serrated setae, that is pennate; the other mouth parts are specific to the genus.

Maxilliped III (fig. 2 *J*) with outer carpal seta outstandingly long and the exognathus with a peculiar phanerotaxia: black terminal denticles with fine hairs between them and, laterally, hairy, unusual phanera.

Chelae symmetrical, anterior peraeopods (II — IV) with a similar morphology: a 3 — 4-dentated propodus on the ventral edge and 2 tubercles on the basipodite (fig. 2 *B*). The three posterior peraeopods with similarly strong claws are slightly spinose and the tubercles mentioned are lacking; the dactylic claws are anteriorly directed.

Uropods short, with a 2-segmented exopodite and a 3-segmented endopodite (no exception in the 5 specimens examined). Uropodal basis with a strong inner, terminal apophysis (fig. 2 *D*).

Male. The phanerotaxia of the body and the morphology of peraeopods and uropods do not differ from those in ♀. The striking features in ♂ are the huge symmetrical chelae (fig. 2 *B*), with a large oval space between the figures (fig. 2 *H*), the more marked crest of the telson (fig. 2 *C*), the two rudimentary pleopods, the presence of aesthetascae in antennules and a very massive genital tubercle.

Uniformly sandy colour, ♂♀ without any pigmented area except for the eyes and the tip of the chela, black (fig. 2 *H*, *J*).

Size : ♂ = 1.8 mm (2, with antennae) ; ♀ = 2.2 mm.

Material, locality : Mediterranean waters of Israel, at Ras-El-Nacura, 28.9.1974, 1 ♂ at the same site on the 4.12.1973, 1 ♂, 2 ♀♀, 1 ♀ juv. and in front of Acre, 20.6.1974, 1 ♂ ; in both localities the species occurred in algae (*Pterocladia* at Acre). But the richest station is T M 15, Bat Yam, 3 km south of Tel Aviv, 1.11.1976, on a subtidal sand-stone platform, in Algae *Jania*, namely 44 individuals ; 8 ♂♂ adults, 5 preadults ♂♂, 3 ♀♀ with eggs ; the rest females nonadult and juvenile. Leg. Prof. Lew

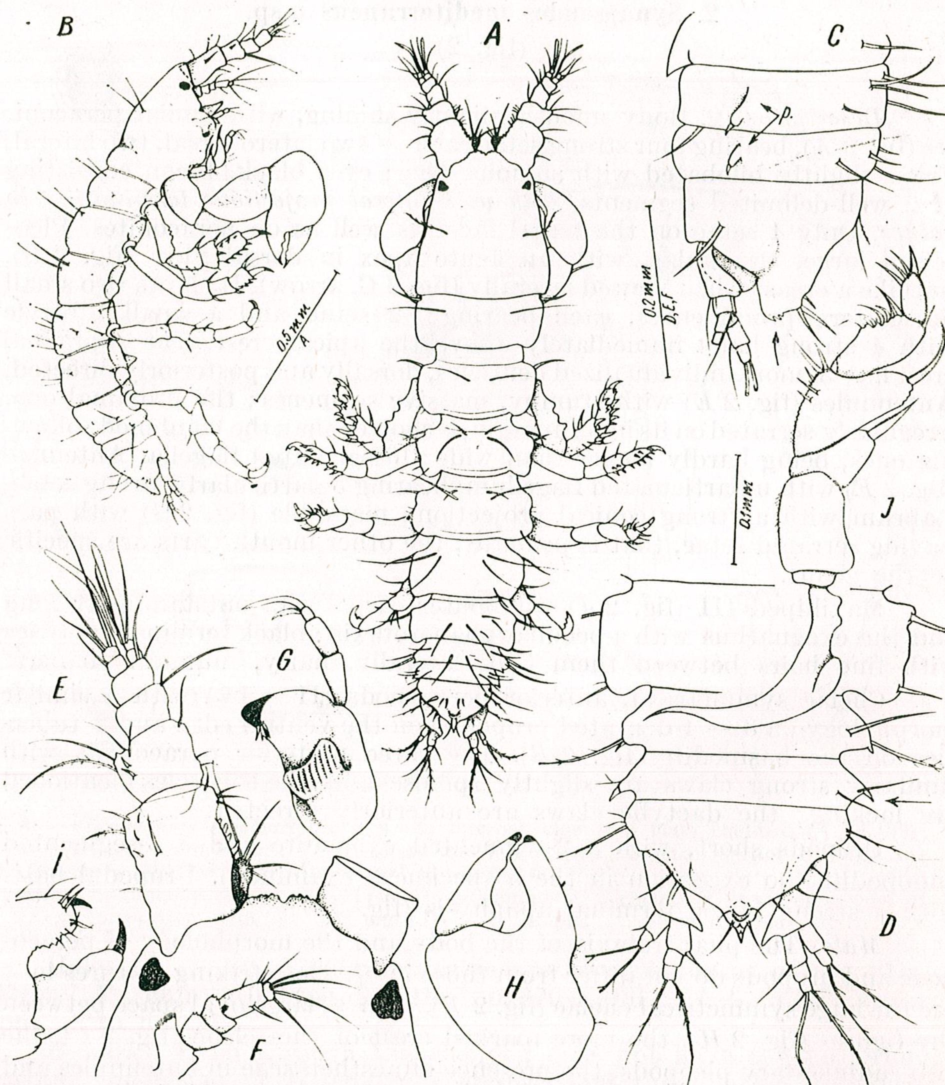

Fig. 2. — *Synapseudes mediterraneus* n.sp. *A*, female, seen from above ; *B*, ♂, in lateral view ; *C*, extremity of abdomen, in lateral view, so as to render evident the rudiments of pleopods (*p*) ; *D*, ditto, seen from above ; *E*, frontal portion of carapace with left antennule (♀) ; *F*, antenna ♀ ; *G*, mandible of chela of a mating ♂ ; *H*, end of chela ♀ ; *J*, maxilliped.

Fishelson. Holotype ♂ = no. 372 in the crustacean coll. of "Gr. Antipa" Museum. Allotype ♀ = no. 373 ibid. and 20 paratypes ♂ ♀, ibid., no. 373 b.

Anatanais dudichi n.sp., *Tanais cavolini* and *Leptochelia dubia* to regularly belong to the species cenosis.

Remarks. *Synapseudes mediterraneus* belongs to the group of species with bilobate rostrum, with symmetrical claws in ♂♂ and with the uropod consisting of 3 segments at the endopodite and 2 at the exopodite ; it is surely a surviving animal of the old inhabitants of the Tethys sea.

Roughly, it mostly resembles *S. comoriensis* Pillai, in which however the shape of the rostrum and of the pleotelson tip is unknown ; it distinctly differs from the latter by the lack of the belts of hairs above the two pleonites. We mention that the number of pleonites in the species *S. comoriensis* made the object of ample comments of Lang ([4], p. 596), then of Gardiner [2]. This was due to the fact that Pillai initially described it as having 5 pleonites, whilst Lang drew only four ; in fact, it has only two pleonites like any typical *Synapseudes*, as it clearly results from the figures I received from the author.

As far as the preliminary description of Riggio [8] allows a comparison, our species differs from the species *S. shiinoi*, the nearest from the geographical viewpoint — the west of the Mediterranean Sea — by the presence of an antennal basal segment short, thick, innerly serrated, with a 2-segmented flagellum, by the lack of the latero-pleonal spines and by the shape of the pleotelson, as well as by the much more gracile antennules in ♀.

From *S. rudis* Menzies, cited by Makkaveeva [5] from the Gulf of Aden, it differs by the symmetry of the claws in ♂ and by other features.

From all the known species it differs in that the pleonites edge bears a spinose apophysis posteriorly directed (Fig. 2 *D*, arrow).

With *S. mediterraneus*, the number of species belonging to the genus *Synapseudes* and known in the World Ocean raises to 14 ; I have not counted, of course, either the 2 species with a single pleotelsonal segment (genus *Curtipleon* mihi, 1977), or *S. idios* Gardiner, 1973 which probably represents a different genus, near to *Metapseudes*, situated — along a phylogenetic line — at the initial end, the extremity of which was reached by *Curtipleon*.

The finding of two representatives of this genus in the Mediterranean Sea fills the gap which existed between the rich fauna of corallicolous *Synapseudes* of the Caribbean Sea and that of the Indian Ocean.

3. **Parapseudes grubei** (Sars)
(fig. 3)

Material : 2 ♀♀, one with 5 eggs (2.1 mm) and 1 juv. (= 1.5 mm) St. 98 Libya 32°07′ N ; 24°06′ E, 70 m, coralloides sand with *Palmophyllum*, 30.09.1975 ; in cenosis : *Leptochelia dubia* (1 ♂, 3 ♀♀), *Leptognatia* sp. (3 ♀♀).

The Libyan specimens correspond in details rather to Sars' figures ([9], pl. 7) than to those given by Lang from Japanese specimens he considered as belonging to the same species [3]. Particularly the epistomal spine, the oblique hyposphenia posteriorly directed parallelly with sternites, the very sharp A_1 and A_2, the mandibular palp and the pleopods are very similar to the former (fig. 3 *D*). The size, the shape of pleotelson and eyes do not correspond; the latter are not so schematically separated as in Sars' figure, they are much larger, nearly globulous, with 10 distinct ommaridia reflecting an intense brown colour. In the Libyan specimens (fig. 3 *A*), aside from the large, intensely coloured eyes, the great length of the cylindrical pleotelson (fig. 3 *C*) and the round (not straight) edges of peraeonites V – VI (fig. 3 *B*) are also striking. It certainly is a matter

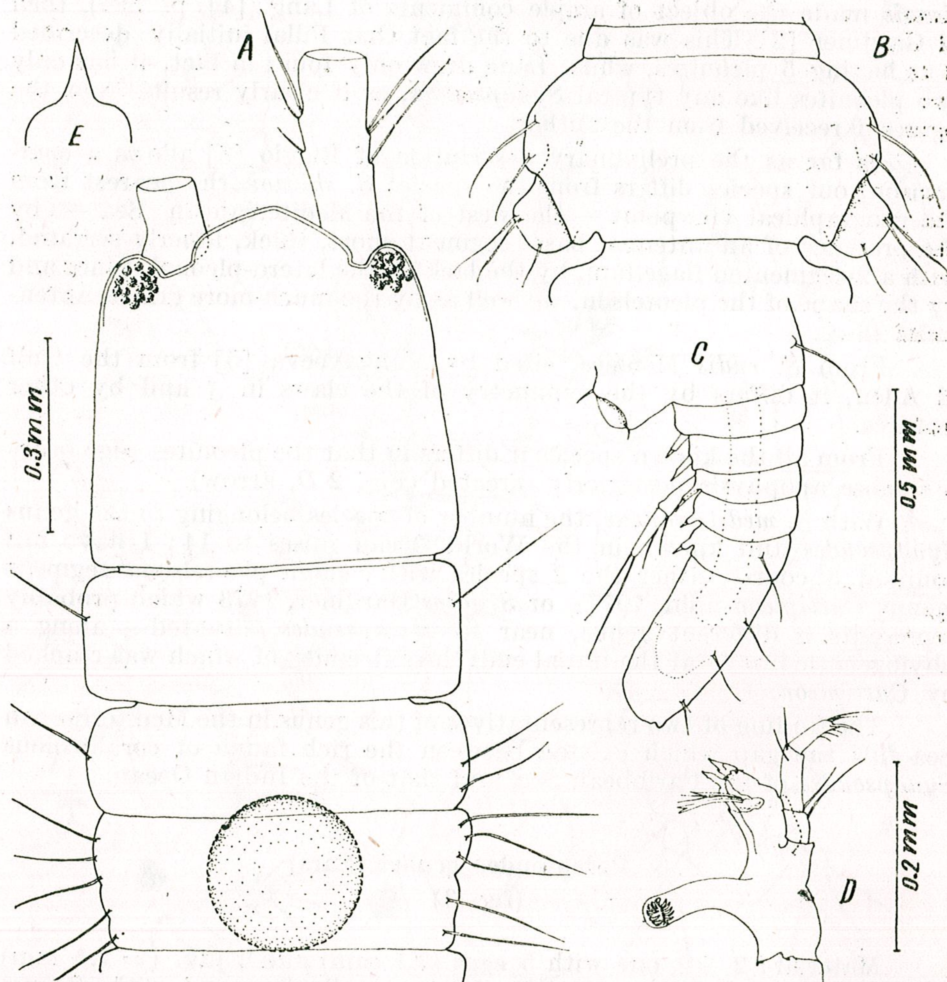

Fig. 3. — *Parapseudes grubei* (Sars). *A*, ♀, seen from above (body slightly flattened by the pressing of the lamella); *B*, free thoracomer V; *C*, pleon in lateral view; *D*, mandible; *E*, epistomal spines.

of local variations which have to be defined in the framework of this species.

Taking into account that the detailed figures given by Lang ([3], figs. 1 – 5) for the Japanese specimens and their description distinctly differ from Grube's genotype by the small size and the shape of eyes, by the richness of setae (particularly on the first abdominal segment); also bearing in mind that the thoracomers are almost equal in size) not distinctly decreasing posteriorly) and considering some microstructural details (all the phanera, not only one of locinia mobili are split at their tips), etc., it results that the Japanese population certainly belongs to another species.

The revision of the genus performed by Lang needs a more thorough study, because it does not seem likely that one and the same species is so widespread: eastern and western Pacific, Hawaii, western Atlantic, Mediterranean and Red Seas, South Africa, even if we think it to be a Tethys relic, as in fact it is.

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LES HESPERIIDAE — LÉPIDOPTÈRES À CARACTÈRES COLLECTIFS

PAR

EUGEN V. NICULESCU

The work points out that family Hesperiidae shows a very interesting mixture of features proper to two suborders (Aparasternia and Parasternia) which are established by the author. These collective features are found both in the thorax and abdomen. The conclusion may be thus drawn that, when classifying the Lepidoptera, family Hesperiidae has to held a taxonomical position occurring between the two suborders.
When introducing the thoracic exoskeleton in a Brassolidae-family species (suborder Aparasternia), the author introduces two new morphological terms.

Par caractères collectifs nous entendons un ensemble de caractères réunis chez un même groupe d'animaux, caractères que nous trouvons aussi séparés chez d'autres groupes où ils constituent des caractères propres à ceux-là. Le groupe possédant des caractères collectifs est intéressant pour la phylogénie et la classification puisqu'il représente l'anneau qui lie entre eux les deux groupes diversifiés.

Les animaux à caractères collectifs sont nombreux parmi les fossiles — surtout dans la classe des Mammifères. La Néontologie nous offre peu d'exemples dont nous présenterons celui découvert par nous dans l'ordre Lépidoptera. La découverte si tardive de ce phénomène parmi les Lépidoptères s'explique par le fait que les lépidoptéristes ont ignoré, presque totalement, l'étude de l'exosquelette. Les quelques études qu'on a entrepris jadis sur cet important appareil des papillons se sont seulement référées à l'aspect purement morphologique du problème. Les travaux publiés par nous sur l'exosquelette sont les seules recherches de *morphologie taxonomique*, travaux de la plus grande importance pour la phylogénie et la classification des papillons.

Vraiment, l'exosquelette nous offre les meilleurs caractères structuraux pour l'établissement des relations phylogénétiques entre familles, superfamilles et sous-ordres [4], [5] et pour la classification de ceux-ci. Pour les espèces et genres l'exosquelette fournit aussi de bons indicateurs phylogénétiques, mais pour ces deux taxa il y a aussi d'autres caractères structuraux offerts par l'armure génitale.

En examinant un grand nombre de papillons (environ 900 espèces) de presque toutes les familles de Lépidoptères, nous avons constaté que l'exosquelette thoracique [5] et abdominal [6], [7] est constitué selon deux types de structure, ce qui nous a permis de diviser l'ordre Lepidoptera en deux sous-ordres [4] : 1. *Aparasternia*, 2. *Parasternia*.

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Voici les caractères généraux des deux sous-ordres [1].

Aparasternia (fig. 1, 2)

1. Le parasternum est absent.
2. Le mesopons, fort sclérifié, allongé antéro-postérieurement, à le bord inférieur droit et l'extrémité bifurquée.
3. La mesophragma est pourvue de processus dorsaux proéminents.

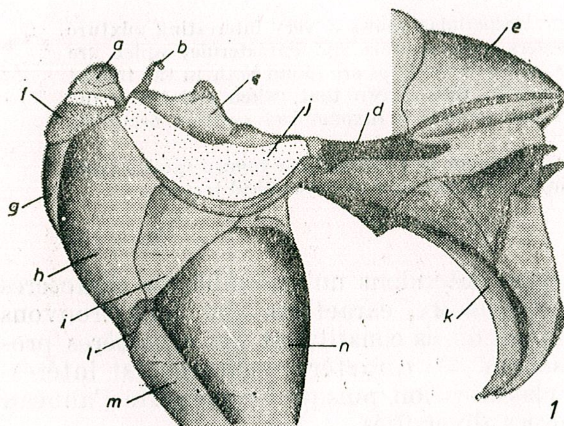


Fig. 1 — *Caligo martia* Godt. : mésothorax, vue latérale. a, mesopraeparapterum ; b, mesoparapterum ; c, mesopostparapterum ; d, mesopons ; e, mesoscutellum ; f, paraperisternum ; g, peristernum ; h, mesepisternum ; i, mesepimerum ; j, membrane mésopleurale ; k, mesophragma ; l, hypomesepimerum ; m, mesocoxae ; n, mesomerum.

4. Le trochantinus est absent.
5. Le premier tergite abdominal culcitiforme (en forme d'oreiller) dépasse en hauteur le bord antérieur du deuxième tergite (fig. 5, 3).

Parasternia (fig. 3, 4)

1. Le parasternum est présent.
2. Le mesopons, faiblement sclérifié, court, à le bord inférieur convexe et l'extrémité postérieure non bifurquée.
3. La mesophragma est lisse et dépourvue de processus dorsaux.
4. Le trochantinus est présent.
5. Le premier tergite abdominal est diversement conformé, mais jamais culcitiforme ; il ne dépasse pas en hauteur le bord antérieur du deuxième tergite, étant situé au même niveau (fig. 5, 1).

Les caractères mentionnés ci-dessus sont très constants à l'intérieur des deux sous-ordres ; ils sont présents chez toutes les espèces et constituent donc des caractères généraux pour chaque sous-ordre. Outre ceux-ci il y a aussi beaucoup d'autres caractères, mais qui sont propres seulement à

[1] Dans ce travail nous avons utilisé la terminologie de Jordan [1], Kusnezov [2] et Niculescu [5], [6], [7]. Les termes morphologiques proposés par nous dans les divers travaux antérieurs sont les suivants : paramesepisternum (1968), subtegulum, mesoparapterum, mesopons, pseudoparasternum, paramesepimerum (1975), mesoparapterum inferior, mesoparapterum superior, mesoparascutum primum, mesoparascutum secundum, mesoparascutum tertium, paramesophragma (1976).

certaines familles ou genres et c'est pourquoi ils ne constituent pas des caractères généraux, ce qui nous a déterminé à les omettre.

Les *Hesperiidae* (fig. 2) présentent un intéressant mélange de caractères collectifs appartenant aux deux sous-ordres ; ce sont les suivants :

1. Le parasternum présent, mais il est partout plus petit que chez les *Parasternia*.
2. Le mesopons est tout à fait du type papilionoïde, ce qui nous a déterminé à placer les *Hesperiidae* parmi les *Aparasternia*.

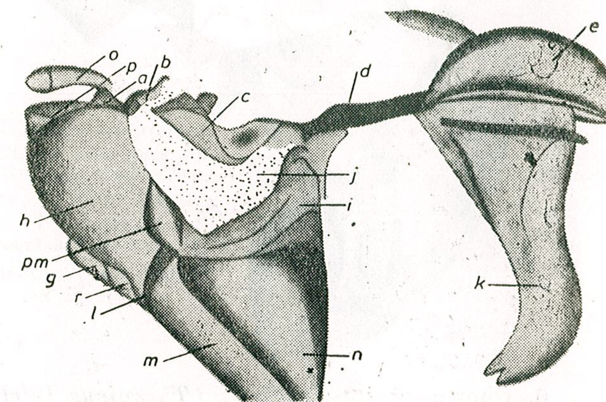


Fig. 2. — *Thespieus lutetia* Hew.(Hesperiidae) : mésothorax, vue latérale. o, subtegulum ; p, parasternum ; pm, protomerum ; r, mesosternum.

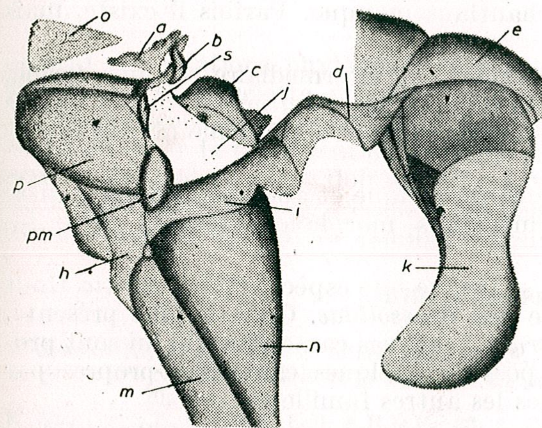
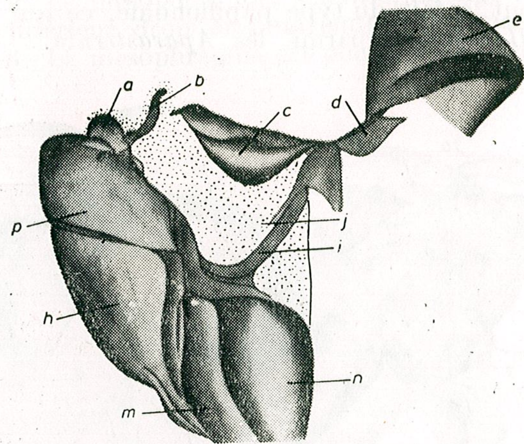


Fig. 3.— *Perisomena caecigena* Kupido : mésothorax, vue latérale. s = paramerum.

3. La mesophragma est du type intermédiaire entre les deux sous-ordres, étant pourvue seulement de petites plaques dorsales (parfois celles-ci manquent aussi), chez certaines espèces ces plaques étant munies de petits processus, mais qui ne sont jamais aussi développées que chez les *Aparasternia*.

4. Il y a un seul mesoparapterum relativement bien développé, tandis que chez la plupart des *Parasternia*, examinés par nous, se trouvent deux sclérites (mesoparapterum inferior et mesoparapterum superior) et chez les *Aparasternia* le seul sclérite est d'habitude très petit et recouvert.

5. Il y a un hypomesepimerum.



F . 4. — *Myelobia smerintha* Hb .(Crambinae), mésothorax, vue latérale. La mesophragma n'est pas figurée.

6. Chez certaines espèces (*Thespieus lutetia* Hew. fig. 2) se trouve un protomerum qui ressemble beaucoup au protomerum existant chez les *Sphingidae* et *Attacidae*, mais chez la plupart des *Hesperiidae* le protomerum est absent comme chez les *Aparasternia*.

7. Le plus souvent le trochantinus manque. Parfois il existe, mais à l'état rudimentaire.

8. Le premier tergite abdominal est intermédiaire, comme forme, entre le tergite culcitiforme qui caractérise les *Papilionoidea* et le tergite des *Parasternia* dont le bord postérieur se trouve au même niveau que le deuxième tergite (fig. 5, 2).

Par conséquent l'exosquelette thoracique et abdominal nous montre que les *Hesperiidae* sont intermédiaires morphologiquement entre les *Parasternia* et *Aparasternia*.

Parmi les exemples choisis se trouve une espèce, *Caligo martia* Godt (fig. 1), appartenant à la famille des *Brassolidae*. Cette famille présente, outre les caractères des *Aparasternia*, quelques caractères qui lui sont propres. D'ailleurs chaque famille possède quelques caractères propres par lesquels elle se distingue de toutes les autres familles.

Nous allons présenter ici deux caractères seulement des *Brassolidae*; il s'agit de deux nouveaux sclérites pour lesquels nous proposons les termes suivants :

1. *Paraperisternum* **nov**. est un sclérite faiblement sclérifié qui se trouve ventralement par rapport au mesopraeparapterum, à côté du peristernum — d'où son nom.

2. *Hypomesepimerum* **nov**. est un sclérite étroit situé entre le mesosternum, mesepimerum, mesomerum et mesocoxae.

Pour conclure nous précisons que l'exosquelette a une importance primordiale surtout pour les catégories supragénériques (famille, superfamille, sous-ordre) où l'armure génitale ne peut fournir aucune indication.

L'exosquelette chez les Insectes a le même rôle que l'endosquelette des Vertébrés et doit, comme ce dernier, être aussi utilisé dans l'établissement des relations phylogénétiques entre les groupes et dans la classification. L'exosquelette des *Hesperiidae*, par le mélange des caractères morphologiques appartenant aux deux sous-ordres, présente un surplus d'intérêt pour la classification générale de l'ordre Lepidoptera. L'établissement de la place qu'on doit accorder à cette famille dans la classification de l'ordre, ainsi que la précision des relations phylogénétiques entre les *Hesperiidae* et les autres familles feront l'objet d'un travail ultérieur.

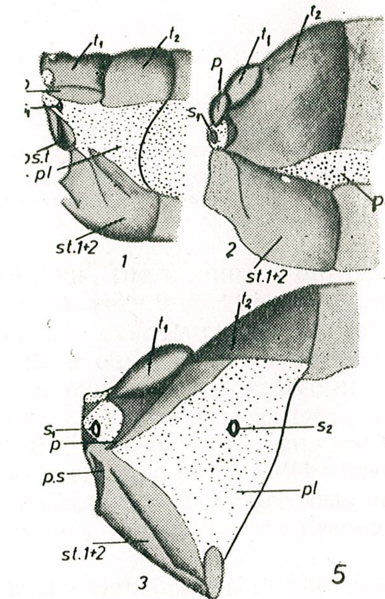


Fig. 5. — Partie antérieure de l'abdomen, vue latérale : 1. *Ascotis selenaria* Den. et Schiff. ; 2. *Astraptes fulgerator* Walch (Hesperiidae) ; 3. *Siproeta trayja* Hbn. (Nymphalidae), t_1, premier tergite ; t_2 IIème tergite ; p, paranotum ; s_1, stigmate du Ier segment ; st. 1 + 2, sternite 1 + 2 ; ps, processus sterniti ; s_2, stigmate du IIème segment ; pl, pleurites ; os.t, ostium tympani.

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THE ZOOGEOGRAPHICAL SIGNIFICANCE OF THE FAUNA OF OLD LAKES

BY

PETRU BĂNĂRESCU

The zoogeographically most significant groups of primary freshwater animals play a minor rôle in the fauna of old lakes; most endemics and species flocks of these lakes belong to secondary freshwater fishes and snails and to peripheral higher crustaceans. It is suggested that these categories are favoured by selection in deep lakes, primary freshwater animals being favoured in rivers.

According to the most authorized opinions, the faunas of old lakes have the following main characteristics: 1. A very high degree of endemism, at the specific, generic and sometimes even suprageneric level. 2. The occurrence of species flocks within either endemic or non-endemic genera. 3. The presence of some species whose closest relatives live in distant areas (for ex. the leech genus *Torix* and the bryozoan *Hislopia* live in Lake Baikal and in South-East Asia), this distribution pattern suggesting the survival in old lakes of archaic, once widely ranging taxa. 4. The freshwater origin of the fauna; old lakes are not remnants of former seas and their few inhabitants of recent marine origin (e.g. the Baikal seal) reached the lakes by continental route.

The present paper deals mainly with the significance of the fauna of old lakes for the general zoogeography of fresh waters, i.e. the understanding of the origin and evolution of freshwater faunas. It therefore refers above all to the third zoogeographical character of these lakes: the occurrence of archaic, formerly widely ranging groups.

The zoogeographically most significant groups of freshwater animals are: primary freshwater fishes, crayfishes (Astacidae, Cambaridae, Parastacidae), true freshwater mussels (Unionacea, Mutelacea), primary freshwater prosobranchiate snails (Bithyniidae, Valvatidae, Pilidae a.o.). These groups are considered as primary freshwater, i.e. they reached their present range exclusively by continental freshwater route. Other zoogeographically highly significant freshwater groups, such as rheophilic insects and the Anostraca, as well as other taxa characteristic of temporary pools cannot be considered here, since they do not live in lakes.

These four zoogeographically significant freshwater groups play a rather minor role in the fauna of old lakes.

Let us consider first Lake Baikal in Siberia, the oldest and deepest in the world and with the highest degree of endemism. Siberia has been an important evolution area for primary freshwater fishes, above all Cyprinidae. Most or all genera the ranges of which are Euro-Siberian or European originated from Siberia, and Siberia was also the dispersal route for a

lot of fishes of East Asian origin from their homeland to Europe [1] — [5]. Many of these fishes became extinct in Siberia during the Ice Age and none of them survived in Lake Baikal. All the 12 species of primary freshwater fishes living in this lake [10] are widely ranging Euro-Siberian, Palaearctic or Holarctic ones. In this respect, the small refuge areas south of Altai Mountains — Talas R. with *Leuciscus* or *Aspiopsis merzbacheri* and West Mongolia with the endemic genera *Oreoleuciscus* and *Acanthorutilus* — are zoogeographically much more significant than Lake Baikal, since they retained something from the Neogene fauna of Siberia.

No crayfishes do presently occur in Siberia, but the close relationships between *Astacus* (Europe) and *Pacifastacus* (weastern North America) [7] proves the former occurrence of Astacidae in Siberia. They did not survive in L. Baikal. The Neogene freshwater mussel fauna of Siberia, too, was much richer than the present one, including u.o. *Unio*, several Margaritiferidae, Amblemidae, etc. [14]. In L. Baikal live only two species of mussels, both widely ranging in Siberia. Better represented in this lake are the primary freshwater prosobranchiates : four endemic and two non-endemic Valvatidae [10], [14]; but the most characteristic prosobranchiates of the lake are the secondary freshwater or mesolimnic Benedictiidae and Baicaliidae.

The primary freshwater fish fauna of Africa is the most complex in the world, including archaic groups (above all osteoglossomorphs), characins and catfishes related to the South American ones and rather recent Asian intruders (cyprinids, mastacembellids, etc.). The three principal lakes of Africa have some endemic species of primary freshwater fishes : Tanganyika has 11 endemic cyprinids, 12 mastacembellids, 15 catfishes, one characin, as against 129 cichlids and 8 — 9 cyprinodontids and peripheral fishes; Malawi (Nyassa) has 14 endemic cyprinids, one mormyrid, one mastacembellid, 13 catfishes, as against some 200 cichlids; Victoria has two cyprinids, two catfishes, as against 164 cichlids and one cyprinodont (all data after Roberts [12]).

These figures seem high comparatively to the total or almost total absence of endemic primary freshwater fishes in lakes Baikal, Ohrid and Titicaca; but they are quite small comparatively to the number of endemic cichlids and other secondary and peripheral freshwater fishes and small even in comparison to the endemic primary freshwater fish species and genera of the main African riverine basins.

No crayfishes live in Africa, but the former occurrence of Parastacidae is probable. They did not survive in the old lakes.

There are some endemic species and even genera, but no species flocks of primary freshwater molluscs in these three lakes : three species of prosobranchiates (Pilidae, Bithyniidae) and eight of mussels in Lake Victoria, an endemic subgenus of Pilidae and six species of mussels on Lake Malawi, an endemic genus of Bithyniidae, several endemic Pillidae and rather many endemic mussels (even five endemic genera) in L. Tanganyika [14]. Much more numerous are the endemic secondary freshwater prosobranchiates, especially in Tanganyika.

Lake Ohrid has an endemic primary freshwater fish (*Pseudophoxinus minutus*), no endemic crayfish, no unionid mussels at all, but five endemic

primary freshwater valvatid snails [13]; much more numerous are endemic species of secondary freshwater prosobranchiates.

The situation is similar with the smallest of the old lakes in the world : L. Titicaca in the Andes, with no endemic species of primary freshwater fishes, crayfishes or mussels and a few prosobranchiate ones.

Curiously enough, primary freshwater fishes and crayfishes include a comparatively high number of endemic species, and even contribute to incipient species flocks in some neither too large, not too old lakes of South-East Asia and New Guinea. In lake Yunnan-fu, China, live two endemic subgenera (five species in all) of the cyprinid genus *Hemiculterella*, while in lakes Paniai and Tigi (southern New Guinea) live five, respectively two endemic crayfish species of *Cherax* [8].

The only lake in which a true species flock of primary freshwater animals developed is the famous, but young (some 10,000 years old) and not too large Lake Lanao, Philippines, with some endemic species of Cyprinidae, belonging to *Puntius* and to four monotypic genera related to the same [6] [9] [11]. In the same lake lives an endemic genus of pilid-snails and several species of secondary freshwater prosobranchiates.

A few of the examples of old lakes endemics mentioned in the literature which have close relatives in distant areas refer to representatives of higher taxa having an old age in fresh waters, no passive dispersal means, being zoogeographically similar to primary freshwater fishes, molluscs, etc. : some families of Oligochaeta (above all Lumbicullidae), leeches, bryozoans, etc. But these are small groups and their significance in the general zoogeography of fresh waters is far below that of fishes, mussels, etc. They likewise represent a small percentage in the fauna of old lakes.

While the four main groups of primary freshwater animals contribute but little to the endemism of old lakes and none of their genera gave birth to species flocks, the higher taxa to which most endemics and all species flocks belong are secondary freshwater or peripheral :

Among fishes : the secondary freshwater Cichlidae in the African lakes, *Orestias* in Titicaca, the Cottocemephorinae and Comephoridae (both offshoots of marine Cottoidei) in Baikal.

Among prosobranchiates : the secondary freshwater (meso- and neolimnic according to Starobogatov [14]) Baicaliidae in Baikal, Pyrgulidae and Lithoglyphidae in L. Ohrid, Littoridinidae in Titicaca, Thiaridae, Lavigeriinae, Paludomidae in Tanganyika and the other African lakes.

Among higher crustaceans : to a certain extent the prawn family Atyidae in the African lakes and especially the Amphipoda : very numerous Gammaridae in L. Baikal, *Hyalella* in Titicaca.

The fact that the numerous endemics of old lakes belong to groups of not too remote marine origin, or are able to cross salt water barriers, is not in contradiction with the fourth zoogeographical character of these lakes : the freshwater origin of their fauna. The secondary and peripheral freshwater groups reached the lakes by ascending rivers, etc., or from the neighbouring riverine net; they are not remnants of seas.

It is not easy to explain why the four main groups of primary freshwater animals play a minor rôle in the fauna of old lakes. Many of their families include, besides a high percentage of reophilic genera and species,

also rather many lacustrine ones. Lacustrine species have evolved even within higher taxa usually considered as typically rheophilic, e.g. schizothoracine minnows and noemacheiline loaches. Crayfishes are the only of the four main groups of primary freshwater animals almost entirely confined to running waters, but even they do include a few lacustrine species (e.g. in New Guinea). Hence, the relative scarcity of endemic species within these four groups in old lakes is not due to their inability to adapt themselves to the lacustrine life.

It must be remembered that from the not too numerous but neither too few primary freshwater fishes endemic to the African lakes, most belong to families having reached this continent only in Pliocene times (Cyprinidae, Mastocembellidae), and quite few to older African families.

These facts prove that primary freshwater fishes, mussels, etc. are able not only to live in lakes, including deep ones, but even to give birth in such lakes to endemic species and small species flocks. However, they do not play an important rôle in the fauna of these lakes, comparatively to secondary freshwater and peripheral groups. The best explanation I find is that secondary freshwater animals benefit from deep lakes, while primary ones from riverine habitats. Lakes receive their fauna from adjacent rivers, in which primary freshwater animals predominate. Lakes are initially dominated by the same groups. The relative abundance of primary freshwater animals in young lakes — Yunnan-fu, etc. and especially Lanao — confirms this assertion. But little by little secondary freshwater and peripheral groups enter the lakes and, having selective advantages, gradually eliminate the primary freshwater ones or prevent their further evolution. This explains why the older a lake, the higher the rôle of secondary freshwater and peripheral groups in its fauna.

It was asserted that primary freshwater fishes, molluscs etc. are the zoogeographically most significant groups in inland waters. These groups dominate in rivers (swiftly of slowly running), shallow lakes, ponds connected to the rivers. The higher taxa that best characterize the great zoogeographical freshwater regions live above all in the riverine net. This net is evolving, sometimes rapidly, allowing the extension or splitting of ranges, e.g. speciation and faunal, evolution. The continuous evolution of the freshwater fauna takes place especially in rivers. Formation of deep lakes is rather accidental. When lakes persist for long periods, active phenomena of speciation and adaptation occur in them. Deep lakes, however, represent "worlds *per se*", without great influence on the fauna of the adjacent riverine net (they receive animals from rivers, but lacustrine species only rarely colonize rivers). Old lakes favour the evolution of secondary freshwater groups and only to a lesser degree that of primary ones and the survival of typical representatives of older faunas.

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LANKESTERELLA BAZNOSANUI NOV. SP. — PARASITE ENDOGLOBULAIRE DE *LACERTA VIVIPARA* L.

PAR

ELENA CHIRIAC et I. STEOPOE

In this paper the authors describe *Lankesterella baznosanui* nov. sp. found in the blood of the lizard *Lacerta vivipara* L. and propose to change the diagnosis of the Lankesterellidae Nöller, 1920 family.

Au cours des recherches faites sur l'ovogénèse nous avons eu l'occasion d'observer une coccidie* dans les capillaires et les veinules de l'ovaire, du corps jaune et des capsules surrénales de lézard *Lacerta vivipara* L. Ainsi, dans les cellules endothéliales des vaisseaux sanguins (en particulier des veinules) nous avons observé des schizontes en divers stades d'évolution. Le schizonte mûr est de grande taille et occupe entièrement la cellule hypertrophiée de l'hôte, dont le noyau est comprimé et rejeté à la périphérie (fig. 1). Aussi, nous avons surpris la schizogonie de type eimérien et même la libération des schizozoïtes, qui sont des éléments allongés aux noyaux sphériques situés au centre, à l'extrémité antérieure accuminée et la postérieure arrondie (fig. 2, 3). Dans quelques cas nous avons observé des schizozoïtes jeunes récemment entrés dans les cellules endothéliales. En jugeant d'après la taille des schizontes mûrs et des schizozoïtes, on peut accepter au moins deux générations agames. La gamogonie avec gamètes mâles et femelles typiques se fait dans les mêmes cellules. Les gamontes ne diffèrent pas entre eux, au commencement; ce sont des éléments allongés, de taille intermédiaire entre les schizontes et les schizozoïtes, à noyau porvu d'un grand nucléole (fig. 4), puis, ils deviennent plus ou moins sphériques et leur cytoplasme se vacuolise. Le gamonte femelle ne change pas essentiellement pour devenir gamète; le gamonte mâle produit un grand nombre de gamètes, tres minces, allongés, aux noyaux postérieurs allongés également. L'oocyste asporé contient 12 à 16 sporozoïtes toujours allongés mais plus gros que les schizozoïtes, un peu en saucisse, aux noyaux arrondis au centre.

Chez les lézards on connait depuis longtemps, grâce surtout à Reichenow [8], les hémogrégarines appartenant au genre *Karyolysus*, mais chez ceux-là la gamogonie et la sporogonie se déroulent chez deux générations de tiques *(Liponyssus saurarum)*, tandis qu'ici le cycle entier paraît évoluer dans le lézard. En plus, ici la schizogonie est nettement eimérienne et les gamètes mâles se forment avant la copula, pendant que chez les espèces de *Karyolysus* (comme chez toutes les hémogrégarines) la schizogonie est de type adeléen, et les gamètes mâles se forment après l'accouplement précoce des gamontes, analogue à la syzygie des grégarines. Chez les lézards on connaît aussi *Schellakia*, dont les espèces sont des Holoeimeriidea typiques, mais à l'encontre de la présente coccidie, chez eux la schizogonie et

* Nous remercions Mme Irène Landau du Musée d'Histoire Naturelle de Paris pour les précieux conseils donnés au cours de nos études.

la gamétogenèse mâle se déroulent dans l'épithélium intestinal et le gamonte femelle évolue dans le tissu conjonctif sous-muqueux [3].

Nous considérons que la coccidie trouvée par nous appartient morphologiquement et biologiquement à la famille Lankesterellidae Nöller, 1920 qui groupe les Holoeimeriidea hétéroxènes à oocyste asporé contenant 12 — 50 sporozoïtes. Les Lankesterellidés sont des parasites des batraciens anoures, des lézards et des oiseaux [1] [2] [3] [4] [6] [9], transmis par les sangsues et les tiques. Le cycle le mieux connu et devenu classique grâce à Noller [5] est celui de *L. minima* parasite de la grenouille verte (foie, rate). La coccidie sanguinicole trouvée par nous se distingue de *L. minima* particulièrement par : 1) la localisation très stricte dans les capillaires et les veinules des ovaires, des corps jaunes et des capsules surrénales ; 2) la spécificité d'hôte, ce dernier étant le lézard *Lacerta vivipara* ; 3) son vecteur qui doit être un invertébré piqueur terrestre, probablement une tique jouant le rôle de transporteur passif des sporozoïtes, tout comme chez *Schellakia*. En tenant compte de tout cela, nous considérons la présente coccidie comme nouvelle en proposant le nom de *Lankesterella baznosanui* nov. sp. en honneur de A. Popovici-Bâznoşanu, biologue roumain qui a étudié les parasites endoglobulaires des batraciens anoures et des tortues aquatiques. En même temps nous proposons de modifier la diagnose de la famille Lankesterellidae Nöller, 1920 de la façon suivante : « Holoeimeriidea hétéroxènes. Oocyste asporé contenant 12 — 50 sporozoïtes. Parasites des batraciens anoures, des lézards et des oiseaux, transmis par des sangsues et par des tiques », au lieu de « Holoeimeriidea hétéroxènes. Oocyste asporé contenant 12 à 50 sporozoïtes. Parasites des Batraciens anoures transmis par des Sangsues » comme elle a été acceptée jusqu'ici [6].

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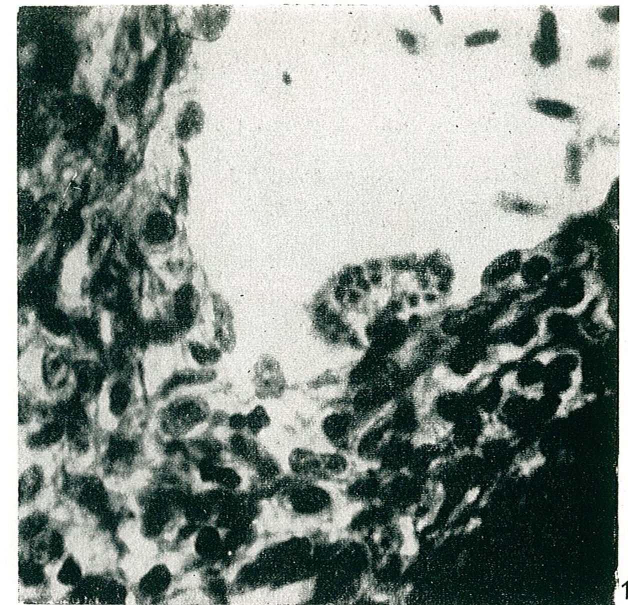
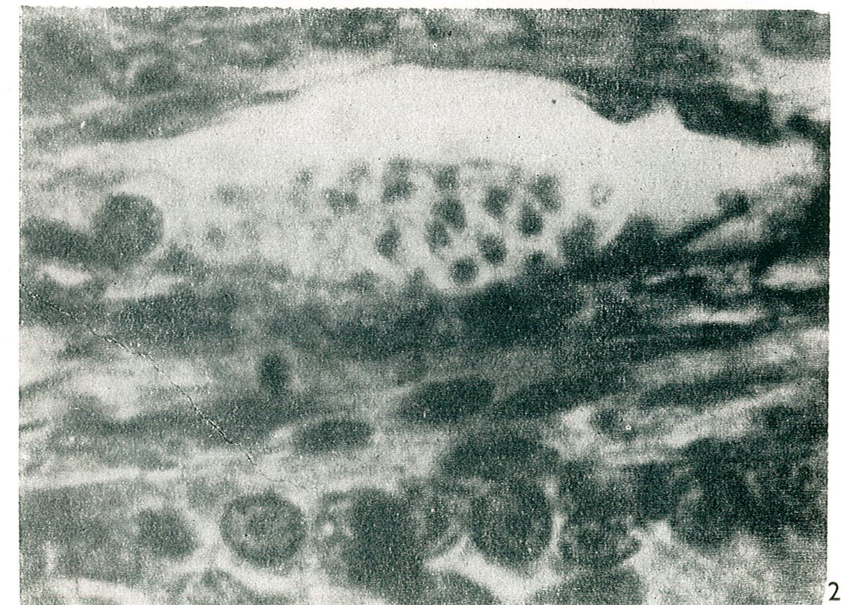
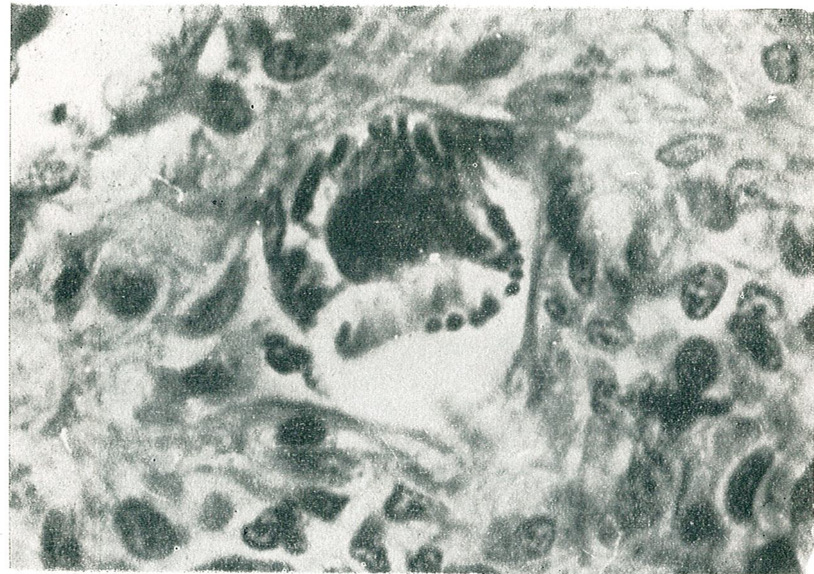




Lankesterella baznosanui nov. sp., 1. — Schizonte mûr ; 2. — schizogonie avec libération des schizozoïtes ; 3. — idem vu à l'immersion ; 4. — gamonte non différencié.



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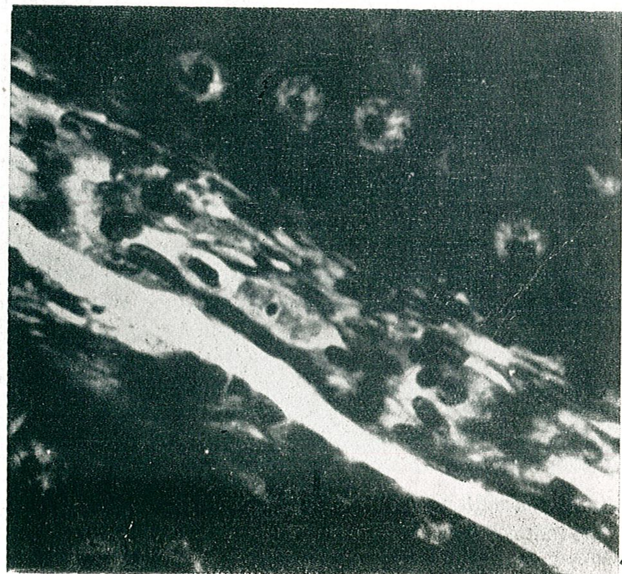

4

CYTOLOGICAL AND BIOLOGICAL ASPECTS OF CELL MIGRATION FROM BLOOD CLOT FRAGMENTS *IN VITRO*

BY

G. ŞANDRU, LIANA ŞANDRU and E. REPCIUC

Cell migration areas obtained by culturing *in vitro* blood clot fragments were represented by erythrocytes and leucocytes in a proportion of about 35 — 40/1. When leucocytes were removed from blood, anticoagulated by EDTA or killed by freezing-thawing before clotting by calcium chloride and fragments preparation the migration areas were strongly depressed. In the presence of antilymphocyte antiserum significant migration inhibitions were obtained according to antiserum dilution. When blood clot fragments belonged to a sensitized donor the migration was inhibited in the presence of the specific antigen. Results were concordant to those obtained by other migration methods performed in parallel.

More than forty years ago Rich and Lewis discovered that the *in vitro* migration of spleen cells of an immune animal were inhibited in the presence of the specific antigen [10]. Since that time the migration inhibition phenomenon has been used as an *in vitro* correlate of delayed hypersensitivity (DH).

Several techniques have been performed to this purpose, such as the spleen explant [10], [12], the capillary tube [6] and more recently the agar techniques : cell migration on agar [11], into an agar plate [4] or from an agar droplet [7]. In a recent paper [13] we have reported a migration technique by using blood clot fragments *in vitro* which has been used for MIF assay in immune guinea-pigs. Further cytological and biological aspects of cell migration from blood clot fragments *in vitro* are presented in this paper.

MATERIAL AND METHODS

Donors : a) C_3H mice immunized by egg albumin (EA) 3×10^{-5} g in complete Freund adjuvant (CFA) 0.05 ml $\times$ 2 subcutaneous inoculations, were sacrificed by ether after three weeks and blood, lymph nodes, spleens harvested ; b) CBA mice immunized by $H_{37}Ra$ mycobacteria containing CFA (Difco) 2 $\times$ 0.05 ml subcutaneously were used as previously described ; c) common guinea-pigs immunized by $H_{37}Ra$ mycobacteria containing CFA (Difco, Mich. USA) were inoculated subcutaneously (2 $\times$ 0.2 ml) and in the nuchal region (0.6 ml). After three weeks blood (i. cardiac puncture), lymph nodes and spleen were harvested ; d) venous blood samples were obtained from healthy adult donors tested for tuberculin sensitivity by i. dermal inoculation of PPD (Cantacuzino Institute).

Migration methods

a) *The blood clot fragment method* was performed as described previously [13]. From a blood clot obtained by incubating 2 ml blood at the room temperature in a 3.2 cm ∅ glass dish, fragments were cut by means of a 1.2 mm ∅ stainless steel trocar. After 4 washings

in TC medium (75 — 100 G) the fragments were cultured in migration chambers for 16 — 20 h. Migration areas (Fig. 1) were microscopically examined and projected on Whatman paper for weighing purposes.

b) *The plasma clot fragment method.* Autologous or homologous plasma prepared by centrifuging blood harvested on EDTA 0.01 M (final concentration) was used as suspending medium for previously prepared blood cells. Peripheral leucocytes or red cells obtained by free sedimentation of EDTA anticoagulated blood were resuspended (45 %) in EDTA-plasma and calcium chloride was added (0.01 M final concentration) with a view to clotting and fragments preparation. Cell-containing clot fragments were incubated under culture conditions and migration areas were examined after 16 — 20 h.

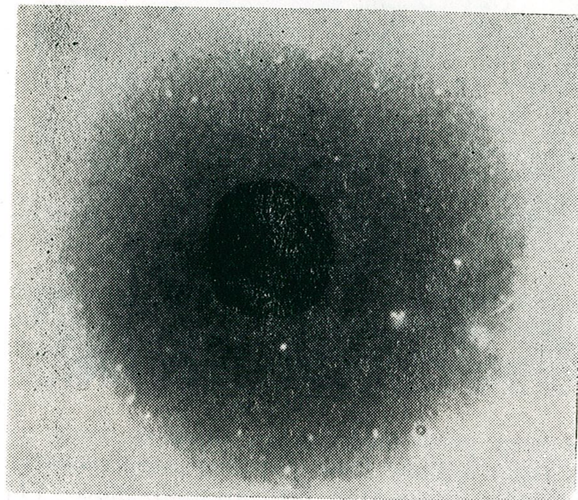

Fig. 1. — A migration area obtained by culturing *in vitro* a human blood clot fragment; magnification × 14.

c) *The capillary tube method* was essentially performed as described by Bendixen and Søborg [1]. Hematocrit capillary tubes (10 cm long, 1.2 mm ∅) were filled up by leucocyte suspension (10^8/ml), heat-sealed, centrifuged 3 minutes (100 G) and cut at cell-fluid interface. The cell-containing fragments were mounted in migration chambers by silicon grease and incubated at 37°C for 20 h under culture conditions.

d) *The spleen explant method* was performed by using a slightly modified Svejcar and Johanovsky technique as described elsewhere [14].

Quantitative Determinations

The migration areas were microscopically examined, photographed and projected on Whatman paper for weighing purposes and quantitatives determinations. Arithmetic mean of 4 weighings, standard deviation and t-test were performed. Migration index (MI) was calculated as percentage of control : MI = (Area X/Area Control) × 100.

Migration chambers were made of glass rings of 1.5 cm int. diameter and 0.15 cm height mounted upon glass plates as described in [13].

Medium and antigens. TC 199 (Difco, USA) containing 0.02 M Hepes and NaOH at pH 7.2—7.4, penicillin 100 U/ml and 10 % foetal calf serum (Gibco, Glasgow, Scotland) were used (control medium). PPD (Statens Institut Copenhagen) and lyophilized BCG (Cantacuzino Institute) were used in a concentration of 10^{-4} and 5×10^{-5} g/ml, respectively (assay medium).

Antiserum against guinea-pig lymphocytes (ALS) was prepared on rabbits and was kindly offered by dr. Boroş from the "D. Danielopolu" Institute for Normal and Pathological Physiology.

Cytological examinations. Migration areas were fixed *in situ* in a methanol-saturated atmosphere and stained by methyl-green pyronin after Una-Papoenheim. Stained preparations were microscopically examined (Ob. Luminar 25 mm/0,15, Ob. 20 ×, Ob. 40 ×) and photographed.

RESULTS

Kinetics of blood clot fragment cells. Following incubation of blood clot fragments under culture conditions the migration of cells started within few minutes. After 6 h incubation the migration mean value represented 60 % of the same areas after 20 h incubation. Microscopical examination of the *in situ* stained preparations showed a large proportion of erythrocytes as against leucocytes, the proportion being 35 — 40/1 (Fig. 2). When plasma clot fragments containing only red cells were

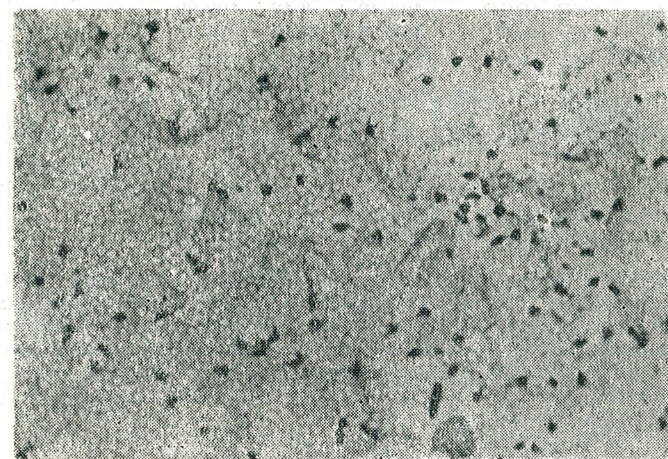

Fig. 2. — Microscopical aspect of the migration area of human blood clot fragment; magnification × 145.

cultured under similar conditions, the migration areas were strongly depressed but still present. If a corresponding number of leucocytes was added to the red cells suspension in EDTA-plasma before clotting by calcium chloride and fragments preparation, the migration index increased again to the control level excepting the case when leucocytes were previously killed by freezing-thawing. When plasma clot fragments containing only buffy coat cells were cultured similarly, the migration areas were two times greater than those obtained by using blood clot fragments from the same donor (Table 1).

Cell migration from blood clot fragments in the presence of antilymphocyte antiserum. In vitro migration of cells from blood clot fragments of guinea-pigs was inhibited in the presence of rabbit anti guinea-pig lymphocyte serum according to antibody concentration. Similar results have been obtained by using the spleen explant method (Fig. 3).

Sensitivity to MIF assay. Migration of cells from blood clot fragments of sensitized donors — mice, guinea-pigs man — was inhibited in the presence of the specific antigen. Sometimes significant inhibitions have been obtained only to the corpuscular antigen (BCG, H_{37}, mycobacteria) and no inhibition or even enhancement of cell migration was found to soluble PPD. When normal blood harvested on EDTA from guinea-pigs was mixed with $1^0/_{00}$ sensitized lymph node cells from H_{37}-immune guinea-

pigs before clotting by calcium chloride and fragments preparation the migration of cells was inhibited in the presence of the specific antigen too (Table 2).

Table I

***In vitro* migration of cells from blood clot fragments**

Donor	Exp. nº.	Method	(cell)	Migration mean ± SD[f]	MI[g]	t-test	p
Man	1	BCF	(BC)[a]	198.42 ± 8.12	100.00		
	2	PCF	(L)[b]	496.92 ± 12.33	250.00		
	3	PCF	(E)[c]	105.39 ± 4.74	53.11	$t^{18}_{1-3} = 9.89$	<0,001
	4	PCF	(E+L)[d]	196.72 ± 7.85	99.14	$t^{12}_{3-4} = 9.95$	<0.001
	5	PCF	(E+kL)[e]	124.68 ± 9.87	64.85	$t^{11}_{3-5} = 2.13$	0.1 > p > 0.05
						$t^{9}_{4-5} = 5.39$	<0.001
Guinea-pig	1	BCF	(BC)[a]	486.18 ± 22.73	100.00		
	2	PCF	(E)[c]	201.36 ± 13.73	41.42		

[a] BCF = the blood clot fragment method for blood cell (BC) migration; [b] PCF = the plasma clot fragment method for leucocyte (L) migration; [c] plasma clot fragments containing erythrochocytes (E); [d] plasma clot fragments containing erythrocytes + leucocytes; [e] plasma clot fragments containing erythrocytes (E) + killed leucocytes (kL) by freezing-thawing; [f] arithmetic mean of 4 weighings of migration areas projections on Whatman paper ± 1 SD (standard deviation) were performed; [g] MI = migration index was performed as percentage of control; (Area X/Area Control) × 100.

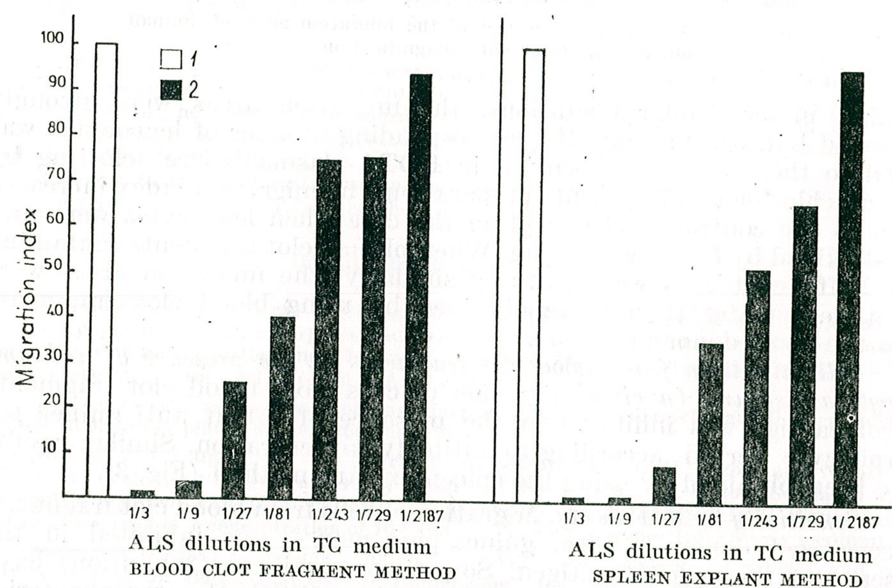

Fig. 3. — *In vitro* cell migration from blood clot fragments and from spleen explants, respectively in the presence of rabbit antilymphocyte serum.

1, cell migration in TC medium + 10 % FCS (control); *2*, cell migration in the presence of antilymphocyte antiserum.

Table 2

***In vitro* antigen-induced inhibition of cell migration from blood clot fragments of sensitized donors**

Donor	No. exp.	Sensitized to :	Assay antigen	BCF[k] Migration	BCF[k] MI	CT[l] Migration	CT[l] MI
Man	3	PPD[a]	No	343.29 ± 13.97		522.31 ± 39.51	
			EA[b]	313.44 ± 22.39	91.30	504.39 ± 32.61	96.57
			PPD[c]	351.60 ± 26.42	102.42	590.70 ± 55.84	113.09
			BCG[d]	240.77 ± 13.14	70.14	371.67 ± 37.56	71.16
						Sp. expl.[m] Migration	Sp. expl.[m] MI
C_3H mice	3	EA (CFA)[e]	No	408.50 ± 10.59		282.42 ± 10.28	
			EA	299.95 ± 9.77	73.43	205.69 ± 8.92	72.83
CBA mice	3	H_{37}Ra Myc.[f] (CFA)	No	748.46 ± 37.57			
			EA	734.51 ± 39.79	98.13		
			PPD	536.44 ± 31.91	71.67	Not tested	
			BCG_{25}[g]	509.91 ± 20.69	68.13		
			BCG_{100}[h]	429.64 ± 21.19	57.40		
Guinea-pig	3	H_{37}Ra Myc.[i] (CFA)	No	317.74 ± 12.35			
			PPD	268.92 ± 14.31	84.63	Not tested	
			H_{37}	182.80 ± 3.72	57.53		
			HSA	318.85 ± 12.72	100.35		
Guinea-pig	2	Normal[j] (BC+LyS)	No	466.63 ± 6.62			
			HSA	392.20 ± 9.28	84.05	Not tested	
			PPD	460.40 ± 17.23	98.66		
			H^{37}	307.22 ± 13.36	65.84		

[a] PPD = sensitized persons have been tested by migration inhibition assay methods; [b] EA = egg albumin, 10^{-4} g/ml in medium; [c] PPD 10^{-4} g/ml; [d] BCG 5×10^{-5} g/ml; [e,f] mice immunized by egg albumin (EA) and H_{37} mycobacteria, respectively; [g,h] BCG 2.5×10^{-5} and 10^{-4} g/ml; [i] guinea-pigs immunized by H_{37} mycobacteria; [j] normal blood cells (BC) were mixed with 1 ‰ sensitized lymph node cells (Lys.) before clotting and fragments preparation; [k] BCF = the blood clot fragment method; [l] CT = the capillary tube method for buffy coat cell migration; [m] the spleen explant method.

DISCUSSION

By culturing *in vitro* blood clot fragments prepared under appropriate conditions significant migration areas could be obtained after a few hours of incubation. The cytological examinations of methyl green pyronin stained preparations showed a large proportion of erythrocytes as against leucocytes. This large proportion of red cells found in the migration areas was in contradiction with their well-known inability to move [9]. However, some *in vitro* experiments have demonstrated the passage of Cr^{51}-labeled human erythrocytes from the peritoneal cavity into the blood stream within a few days [5]. When plasma clot fragments containing red cells were cultured *in vitro* the migration areas were still present although strongly depressed. Thus the migration of red cells appears as depending on the presence of viable leucocytes even in a small proportion within the migration areas. When leucocytes of blood clot fragments

were previously killed by freezing-thawing the migration of the remaining red cells strongly decreased, as seen in table 1. In the presence of the anti-lymphocyte serum previously absorbed on homologous erythrocates, the migration of cells from blood clot fragments was significantly inhibited according to antiserum dilution and similarly with spleen cell migration from explants cultured identically. When blood clot fragments of sensitized donors were cultured in the presence of the specific antigen, significant migration inhibition values were obtained which were concordant to those obtained by other migration methods performed in parallel [13]. Experiments have demonstrated that lymphocytes existing inside the blood clot fragment ensured the antigen-induced inhibition of blood cell migration. When 1 $^{0}/_{00}$ sensitized lymph node cells were added to normal blood harvested on EDTA before clotting by calcium chloride and fragments preparation, the migration of cells was inhibited in the presence of the specific antigen too. This situation is quite similar to that described for the macrophage migration system when a few sensitized cells were able to ensure the antigen-induced migration inhibition of a great number of normal macrophages [3] [5]. Since 1 % of the whole lymphocyte population is really sensitized by an antigen we may suppose that a few sensitized lymphocytes existing inside the blood clot fragment are sufficient to ensure the antigen-induced inhibition of cell migration when they belong to an immune donor.

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L'ACTIVITÉ DE QUELQUES ENZYMES DE LA MUQUEUSE INTESTINALE CHEZ *GOBIUS CEPHALARGES* ET *GOBIUS MELANOSTOMUS* EN FONCTION DE LA SALINITÉ

PAR

LOTUS MEŞTER, DRAGOŞ SCRIPCARIU et RADU MEŞTER

The activity of acid phosphatase, alkaline phosphatase and nonspecific esterase in the intestinal tract of two species of fish (*Gobius cephalarges* et *Gobius melanostomus*) was studied, using histochemical methods. The histochemical observations indicate, that alkaline phosphatase of fishes from marine medium appear with an increased activity. Acid phosphatase and nonspecific esterase were heterogeneous distributed along the whole length of the epithelium of the intestinal tract.

Parmi les structures osmorégulatrices intervenant dans les processus de l'absorption de l'eau, des ions et des substances organiques, le tube digestif des poissons occupe une place assez importante. La signification fonctionnelle de la distribution et de l'activité des enzymes dans l'epithélium intestinal des poissons, par rapport aux processus osmorégulateurs, est fort peu connue [2] [4] [6] [10]. Les études antérieures ont mis en évidence une activité accrue de la phosphatase alcaline et de l'ATP-ase intestinale, suite à l'adaptation de certains poissons d'eau douce ou euryhalins, à l'eau de mer [3] [6] [8]. On a suggéré l'intervention de ces enzymes dans les mécanismes aidant l'absorption de l'eau et des sels minéraux, chez les poissons vivant de façon temporaire ou permanente, en milieu marin.

Dans le présent travail, nous nous sommes proposé d'étudier l'activité de la phosphatase alcaline, de la phosphatase acide et de l'estérase de la muqueuse intestinale, chez deux espèces de gobiidae euryhalines

MATÉRIEL ET MÉTHODE

Les recherches furent effectuées sur deux espèces de Gobiidae euryhalines, *Gobius cephalarges* Pallas et *Gobius melanostomus* Pallas, collectés dans le Mer Noire et le lac Razelm (lac saumâtre à salinité variable entre 1 et 4 $^{0}/_{00}$). Le tube digestif des animaux a été fixé en acétone, au froid ; les pièces furent déshydratées par le benzène et incluses en paraffine à 50°C. On a effectué parallèlement des sections sur des préparations au cryostat.

Sur des sections histologiques de 8 µ on a mis en évidence les enzymes : la phosphatase alcaline et la phosphatase acide selon la technique décrite par Burnstone [1], en utilisant l'alpha-naphtyl-phosphate comme substrat et les sels de diazonium Blue BB et Fast Violet LB comme agent de couplage ; l'estérase non spécifique, avec alpha-naphtyl-acétate comme substrat, selon la technique décrite par Pearce [5]. On a étudié comparativement l'intensité de coloration des sections histologiques de différentes portions du tube digestif.

RÉSULTATS

La phosphatase alcaline. Ainsi qu'il ressort de la Planche I, sur la base de la réaction de coloration histochimique, l'activité de la phosphatase alcaline apparaît plus accrue dans la muqueuse du tube digestif chez les deux espèces de poissons qui vivent en milieu marin. Les observations histologiques ont révélé que chez la même espèce, collectée dans le même biotope, la distribution de la phosphatase alcaline apparaît relativement uniforme tout le long du tube digestif. Chez *Gobius cephalarges* du milieu marin, la réaction enzymatique est intense et même très intense dans la région antérieure et respectivement postérieure de l'intestin (Pl. I B, D).

L'étude comparative de la distribution de l'activité enzymatique chez les deux espèces de Gobiidae du milieu marin révèle une activité plus intense de la phosphatase alcaline dans la muqueuse intestinale chez *Gobius cephalarges*, que chez *Gobius melanostomus.* Tant chez *Gobius cephalarges* que chez *Gobius melanostomus* du milieu saumâtre, l'activité de la phosphatase alcaline intestinale présente une réaction de coloration bien faible ou presque inexistante, tout le long de l'intestin (Pl. I A, C).

La phosphatase acide. L'étude de l'activité de la phosphatase acide sur des préparations histologiques nous a permis de constater une distribution assez hétérogène chez les deux espèces de poissons adaptés aux milieux à salinités variables. Chez *Gobius melanostomus* pêchés en eau saumâtre, la phosphatase acide intestinale présente une réaction de coloration plus intense que chez la même espèce provenant du milieu marin (Pl. II A, B). On a constaté les mêmes différences chez *Gobius cephalarges* (Pl. II C, D).

Les différences concernant l'activité enzymatique ne sont pas significatives, parce que la distribution de l'enzyme n'est pas uniforme au long du tube digestif et les variations en intensité de la coloration ne sont pas les mêmes chez tous les exemplaires. Les variations dans l'activité de la phosphatase acide peuvent être influencées par d'autres facteurs physiologiques ou de milieu, différents de ceux osmotiques.

L'estérase. Cette enzyme présente une répartition hétérogène le long du tube digestif des espèces investiguées. Chez *Gobius cephalarges*, l'enzyme est bien mise en évidence et différente en fonction du milieu de vie : les poissons d'eau saumâtre présentent une activité enzymatique intestinale intense et uniforme, autant dans la région antérieure que dans celle postérieure de l'intestin (Pl. I E). Chez les poissons d'eau marine, l'enzyme présente une activité assez faible, en comparaison de la même espèce provenant du milieu saumâtre (Pl. I F).

Chez *Gobius melanostomus*, les variations de l'activité enzymatique sont différentes dans l'intestin, dans le cadre du même biotope : chez les poissons d'eau saumâtre, la région antérieure de l'intestin présente une activité estérasique plus intense que la région postérieure ; chez les espèces marines, la répartition enzymatique est inverse (Pl. II E, F).

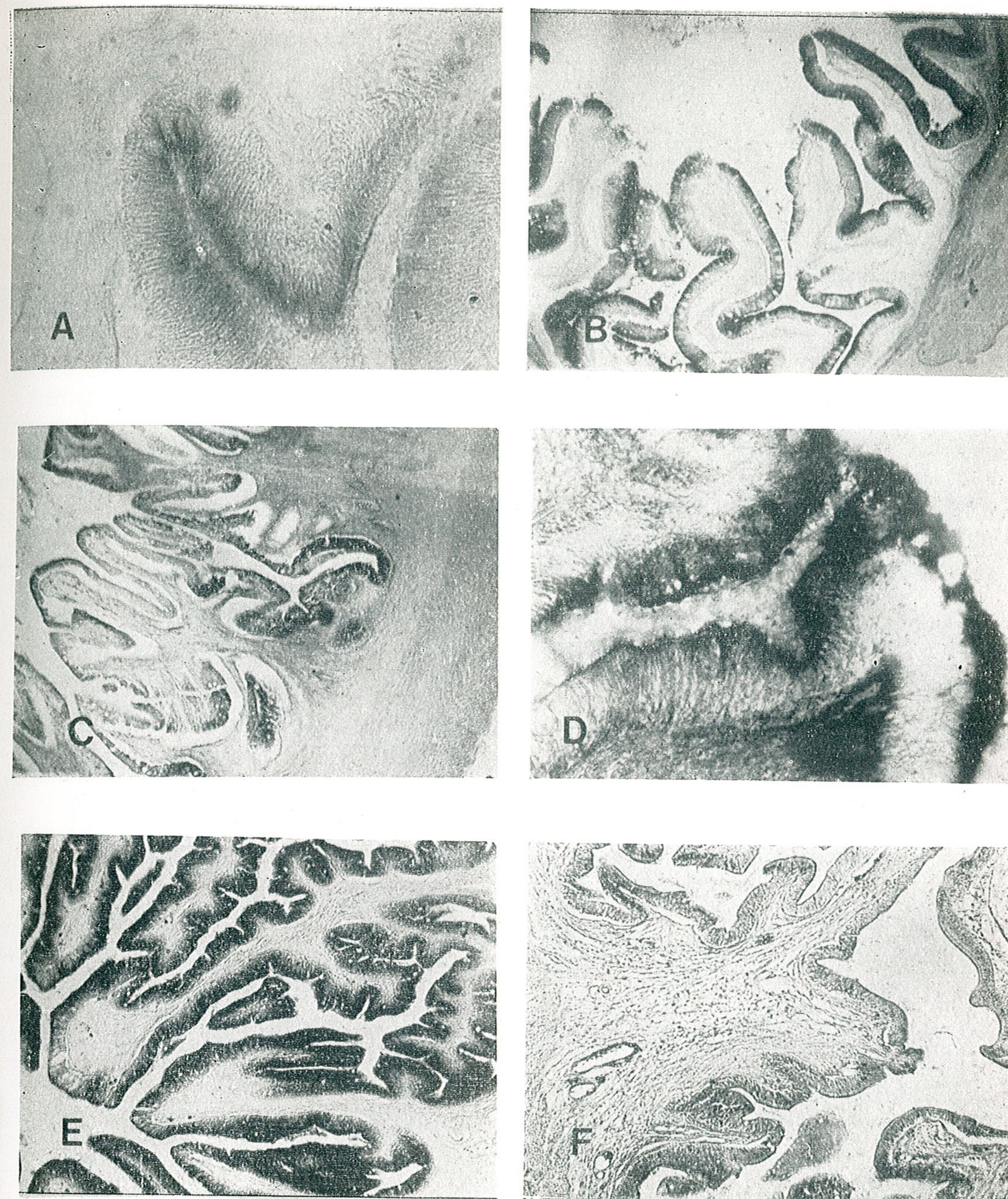

PLANCHE I

A, La phosphatase alcaline dans l'intestin antérieur de *Gobius cephalarges*, adapté à l'eau saumâtre.
B, La phosphatase alcaline dans l'intestin antérieur de *Gobius cephalarges*, adapté à l'eau marine.
C, La phosphatase alcaline dans l'intestin antérieur de *Gobius melanostomus*, adapté à l'eau saumâtre.
D, La phosphatase alcaline dans l'intestin postérieur de *Gobius melanostomus*, adapté à l'eau marine.
E, L'activité estérasique non spécifique dans l'intestin postérieur de *Gobius cephalarges*, adapté à l'eau saumâtre.
F, L'activité estérasique non spécifique dans l'intestin postérieur de *Gobius cephalarges*, adapté à l'eau marine.

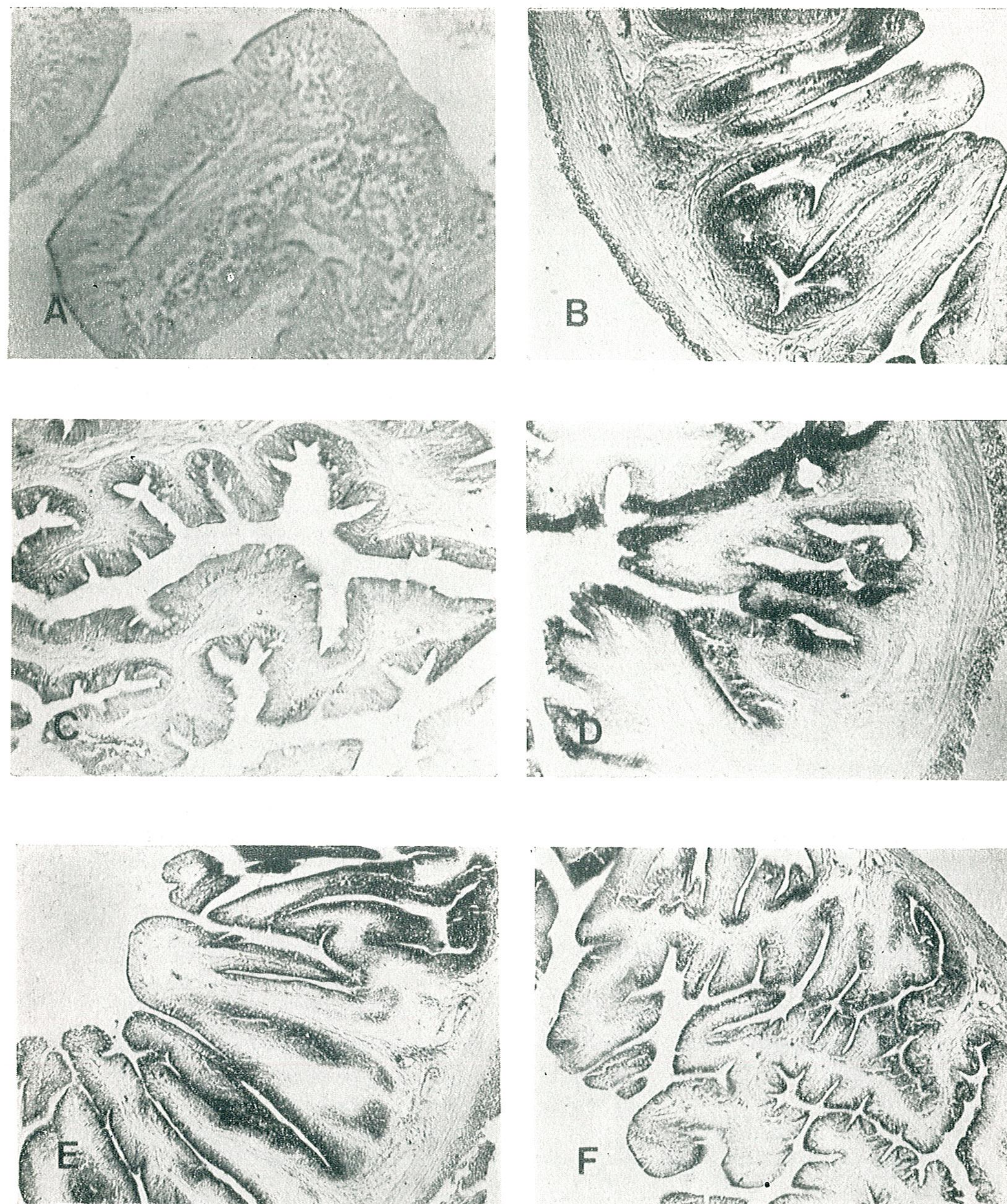

PLANCHE II

A, La phosphatase acide dans l'intestin postérieur de *Gobius melanostomus*, vivant en eau saumâtre.
B, La phosphatase acide dans l'intestin postérieur de *Gobius melanostomus*, vivant en milieu marin.
C, La phosphatase acide dans l'intestin antérieur de *Gobius cephalarges*, vivant en milieu saumâtre.
D, La phosphatase acide dans l'intestin postérieur de *Gobius cephalarges*, vivant en milieu marin.
E, L'activité estérasique non spécifique dans l'intestin antérieur de *Gobius melanostomu* adapté à l'eau saumâtre.
F, L'activité estérasique non spécifique dans l'intestin antérieur de *Gobius melanostomus*, adapté à l'eau marine.

DISCUSSIONS

Il est bien connu le fait que dans les conditions d'eau de mer, autant les poissons euryhalins que ceux marins absorbent par leur intestin une partie de l'eau et des sels minéraux. Les études d'Utida [8], Utida et Isono [9], Utida et coll. [10]; Oide [7], effectuées sur la truite et l'anguille, ont démontré une activité accrue de la phosphatase alcaline intestinale, chez les poissons adaptés temporairement ou vivant en milieu marin. Nos recherches sur les gobiidae, corrélées aux données de la littérature, nous permettent d'étayer l'idée selon laquelle l'activité accrue de la phosphatase alcaline des cellules de la muqueuse intestinale des poissons adaptés au milieu marin apparaît comme un mécanisme compensateur relié au processus osmorégulateur. Ainsi qu'avancé par Oide [7], l'activité de la phosphatase alcaline intestinale chez les poissons adaptés au milieu marin est intensifiée par la présence de NaCl, tandis que l'absorption de l'eau et des ions, par le degré d'alcalinité du milieu intestinal.

En ce qui concerne les deux autres enzymes étudiées (la phosphatase acide et l'estérase), nos données histo-enzymologiques ne permettent aucune corrélation entre ces dernières et leur activité dans les processus osmorégulateurs. Les estérases non spécifiques des cellules de la muqueuse intestinale peuvent avoir de multiples fonctions : sécrétrices, de résorption et de synthèse. Leur répartition variée dans la muqueuse intestinale suggère leur importance toute particulière dans la synthèse (ou la lyse) de certains esters, par rapport à la biosynthèse des membranes lipoprotéiques ou au processus de sécrétion, processus à plus grande intensité chez certaines espèces de poissons vivant en milieu saumâtre.

Les fonctions métaboliques cellulaires de la phosphatase acide sont multiples et controversées. La présence de la phosphatase acide dans les cellules de la muqueuse intestinale de certains poissons peut refléter certains besoins énergétiques cellulaires.

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ACTION OF HYDROCORTISONE HEMISUCCINATE ON THE INCORPORATION OF ^{3}H-THYMIDINE INTO DEOXYRIBONUCLEIC ACID OF SOME A2G MICE ORGANS

BY

A. D. ABRAHAM, MARIA BORŞA and Z. URAY

The process of DNA biosynthesis and retention of ^{3}H-thymidine-labelled DNA in some A2G mice organs has been studied after administration of hydrocortisone hemisuccinate (HCHS). The results indicated the stimulation of biosynthesis and retention of DNA in thymus after administration of HCHS, in contrast with the spleen, where inhibitory effects were observed. HCHS did not influence these processes at the level of liver and small intestine.

The effect of stress-agents is mainly exerted by ACTH stimulation of glucocorticoid hormones secretion [15], [16], which induces metabolic changes at the level of some organs, especially in liver and lymphatic organs [2], [6], [11]. By their interaction with intracellular receptors, especially nuclear receptors, these hormones regulate nucleic acid and protein metabolism [3] [4] [14].

The reason of hormones penetration into cell nuclei from extracelullar space may be to modify the activity of some genes, which influences cell metabolism and proliferation [7] [9].

In the present work the action of hydrocortisone hemisuccinate (HCHS) on the incorporation of ^{3}H-thymidine into DNA of thymus, spleen, liver and small intestine and the retention of labelled DNA were studied.

MATERIAL AND METHODS

Two hundred A2G mice weighing 25 – 35 g were used. The animals were kept under standard conditions and 18 hours before killing they received no food. The animals were injected i.p. with 40μCi methyl-^{3}H-thymidine (per 100 g of body weight) dissolved in 0.9 per cent NaCl solution. The specific activity of radioactive labelled substance was 5 Ci per mmol (purchased from R. C. Amersham). Fifty mice received i.m. injections containing 1 mg and another 50 animals – 5 mg HCHS dissolved in a mixture of methylglycine and natrium-hydrocarbonate solution (purchased from Uzina de Medicamente Bucureşti).

The determination of DNA was made using a technique described previously [1]. The specific radioactivity of labelled DNA was determined after the neutralization of DNA extracts by 1 N KOH. The samples were mixed with 0.5 ml hyamine hydroxide (Nuclear Enterprise, Chicago) and 10 ml of Bray's solution. The radioactivity of samples was measured at 10°C by means of a Betaszint BF-5003 scintillation spectrometer.

RESULTS

^{3}H-thymidine is an excellent precursor of DNA biosynthesis, which incorporates only into *de novo* synthesized DNA of living cells. As reference

value the specific radioactivity of DNA was used, obtained one hour after injection of ^{3}H-thymidine.

Our results indicated that the administration of 1 mg or 5 mg HCHS significantly increased the specific radioactivity of thymus DNA after 24 and 48 hours (Table 1), but 5 mg HCHS after 72 hours determined significant decrease of DNA retention. Comparing the specific radioactivity of DNAs extracted from thymi of animals killed after different periods against controls killed after the same periods, an increase of the specific radioactivity of DNA (especially after 24 and 48 hours) was observed.

Table I

Incorporation of ^{3}H-thymidine into thymus DNA and retention of labelled DNA after administration of HCHS. (The results represent the mean values of specific radioactivity of DNA in DPM per mg DNA).

Time (h)	1	3	10	24	48	72
Control groups						
$\bar{X}$	2 118	1 931	1 916	1 837	1 265	1 020
S.E.	±89	±145	±127	±157	±120	±146
n	8	6	6	6	6	6
PC_1	100	91.2	90.5	86.7	59.7	48.2
P_1	—	>0.05	>0.05	>0.05	<0.001	<0.001
1 mg HCHS						
$\bar{X}$		1 953	2 068	2 793	2 649	1 852
S.E.		±126	±91	±159	±235	±174
n		5	6	5	5	5
PC_1		92.2	97.6	131.9	125.1	87.4
P_1		>0.05	>0.05	<0.01	<0.05	>0.05
PC_2		101.1	107.9	132.2	209.4	181.6
P_2		>0.05	>0.05	<0.001	<0.001	<0.01
5 mg HCHS						
$\bar{X}$		2 414	2 388	2 939	2 651	1 105
S.E.		±229	±152	±235	±193	±138
n		5	5	5	5	5
PC_1		114.0	112.8	138.8	125.2	52.2
P_1		>0.05	>0.05	<0.01	<0.05	<0.001
PC_2		125.0	124.6	160.2	209.6	108.3
P_2		>0.05	>0.05	<0.01	<0.01	>0.05

$\bar{X}$ = mean value, S.E. = standard error, n = number of animals.

PC_1 = per cent difference between the mean value of control group sacrificed after 1 h and the mean values of other control groups or treated groups.

PC_2 = per cent difference between the mean values of control groups sacrificed at different times and treated groups sacrificed at the same time.

P_1 and P_2 = significance of differences.

In contrast with the thymus, in the case of spleen the incorporation of ^{3}H-thymidine into DNA and the retention of labelled DNA decreased already after 10 hours (Table 2). Our experiments concerning the liver and small intestine showed that the administration of 1 mg or 5 mg HCHS did not influence the biosynthesis of DNA or the retention of labelled DNA.

Table 2

Incorporation of ^{3}H-thymidine into spleen DNA and retention of labelled DNA after administration of HCHS. (The results represent the mean values of specific radioactivity of DNA in DPM per mg DNA)

Time (h)	1	3	10	24	48	72
Control groups						
$\bar{X}$	2 327	1 970	2 297	2 137	1 443	1 223
S.E.	±158	±147	±259	±175	±215	±141
n	7	5	5	5	5	5
PC_1	100	84.7	98.7	94.8	62.1	52.6
P_1		>0.05	>0.05	>0.05	<0.01	<0.001
1 mg HCHS						
$\bar{X}$		1 972	1 803	1 542	1 676	1 358
S.E.		±147	±129	±130	±133	±41
n		5	5	5	5	6
PC_1		84.7	77.5	66.3	72.0	58.4
P_1		>0.05	<0.05	<0.02	<0.02	$<0,001$
PC_2		100.1	78.5	72.2	116.0	111.0
P_2		>0.05	>0.05	<0.02	>0.05	>0.05
5 mg HCHS						
$\bar{X}$		2 024	1 967	1 828	1 193	1 206
S.E.		±200	±170	±194	±130	±235
n		5	5	5	5	5
PC_1		87.0	84.3	78.6	51.3	51.8
P_1		>0.05	>0.05	<0.05	<0.001	<0.001
PC_2		102.7	83.6	85.5	82.6	98.6
P_2		>0.05	>0.05	>0.05	>0.05	>0.05

The explanation is the same as for table 1.

DISCUSSION

There are different opinions concerning the action of glucocorticoid hormones on the biosynthesis and degradation of DNA in lymphatic organs. Some authors consider that these hormones are antimitotic, inhibiting DNA biosynthesis in the thymus and lymph nodes [12] [17]. Lang and co-workers [10] suppose that hydrocortisone acts at the level of DNA biosynthesis only in the case of lymphocytes which show pycnotic or cytoplasmolytic aspects. Other data show that glucocorticoid hormones influence cell division in prophase or in the final part of interphase, increasing the activity of adenylate cyclase and the concentration of cyclic AMP [19].

Autoradiographic experiments showed that only medium and large thymic lymphocytes incorporate ^{3}H-thymidine into their DNA, while small lymphocytes have no or little capacity to use this precursor in DNA biosynthesis [5] [8] [13].

Our results indicate an increase of ^{3}H-thymidine incorporation into thymic DNA 24 hours after administration of HCHS, which suggests either an increased DNA synthesis at the level of medium and large lymphocytes, or an increase of the number of labelled cells in the thymus

from extrathymic tissues. In contrast with the results obtained in the case of thymus the action of HCHS at the level of spleen manifests itself by inhibition of DNA biosynthesis and retention of labelled DNA. These observations are in agreement with the well-known lymphocytolytic effect of glucocorticoids [5] [18].

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THE pH INFLUENCE ON THE PROCAINE ACTION AT THE MUSCLE FIBER MEMBRANE LEVEL

BY

ŞTEFAN AGRIGOROAEI and ION NEACŞU

The blocking action of procaine at pH = 7.2 is a complex phenomenon. One of its components, localized in the outer membrane layer, is the membrane hyperpolarization in the resting state, contributing to a rise of the excitation threshold. Another one is the membrane phospholipid sols stabilization in the inner layer by the neutral procaine.

At pH = 6 the anesthetic produces two different effects with different onset thresholds in respect to drug concentration : a small membrane hyperpolarization at 2.5 mM, and only a depolarization at the lower concentrations. Both these phenomena occur in the outer membrane layer.

There are several investigations concerning the procaine action at a normal or alkaline external pH [4], [5], [18], but fewer regarding its action at an acid external pH. It is well known that in an acute infection procaine cannot exert its blocking effect [6]. An explanation for this fact was forwarded invoking the acid local pH, when procaine is present almost entirely in the cationic form [2] and therefore unable to penetrate into the membrane structure [14].

We consider however that the procaine blocking action is a complex phenomenon, each of its components having specific sites in the membrane structure and proper conditions of achievement. In this respect, the "2-M.S.I." theory [1] offers a bassis for the interpretation of biophysico-chemical mechanisms of the local anesthetic action.

MATERIAL AND METHODS

The experiments were conducted on the frog (*Rana ridibunda*) sartorius fiber membrane, using glass intracellular microelectrodes technique. The pH of Ringer solution was maintained alkaline (7.2) by a bicarbonate buffer, and acid (pH = 6) by a phosphate buffer.

In a series of experiments, at an alkaline pH (7.2), the procaine solution was prepared by adding the drug in an anesthetic (2.5 mM) or an underanesthetic (1 mM) concentration [14] to the normal Ringer. In another series, procaine was added by substituting an equimolar amount of K^+ ions in the physiological solution. A control experiment with 1 mM K^+ substituted for 1 mM Na^+ was likewise carried out.

At an acid pH (= 6), the procaine solutions were prepared by adding the drug in an amount of 2.5, 1 and 0.5 mM to the normal Ringer.

RESULTS

The resting potential values recorded in our experiments were within 90.62 mV and 93.62 mV range (SE about 1) (Figs 1 — 7 : RP).

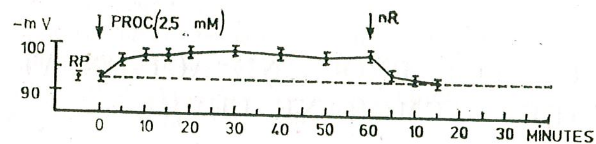

Fig. 1. — The effect of 2.5 mM hypertonic procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 7.2.

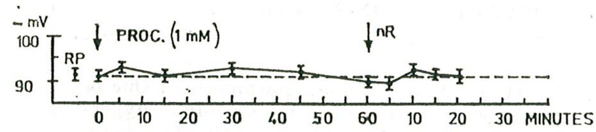

Fig. 2. — The effect of 1 mM hypertonic procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 7.2.

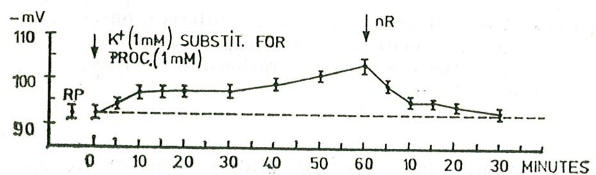

Fig. 3. — The effect of 1 mM isotonic procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 7.2.

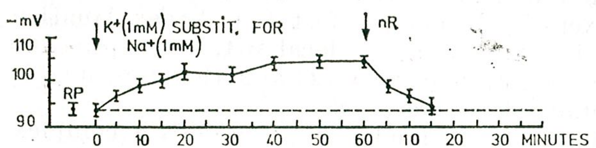

Fig. 4. — The resting potential of the frog sartorius muscle fiber membrane in a 1 mmol/l K^+ substituted for 1 mmol/l Na^+-Ringer solution, at pH = 7.2.

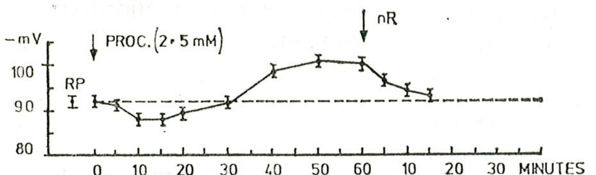

Fig. 5. — The effect of 2.5 mM procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 6.

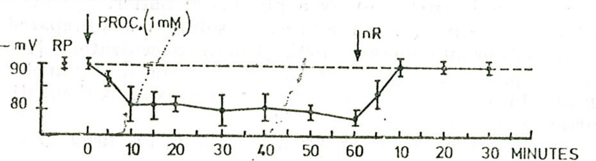

Fig. 6. — The effect of 1 mM procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 6.

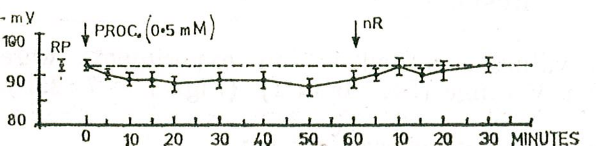

Fig. 7. — The effect of 0.5 mM procaine on the resting potential of the frog sartorius muscle fiber membrane, at pH = 6.

In the alkaline medium (pH = 7.2), when the normal Ringer was replaced for a procaine-Ringer solution, a membrane hyperpolarization was observed, varying in the same direction with the drug concentration. The hyperpolarization produced by 2.5 mM procaine was of 4.69 mV (Fig. 1). Values of the same order of magnitude have been obtained on other biological membranes as well [12]. In the case of a 1 mM procaine-Ringer solution, a very weak hyperpolarization (0.25 mV) was found (Fig. 2). The onset of hyperpolarization was fast enough, but the potential recovery at the normal value, when the fibers were replaced in a normal Ringer, was slower (Figs. 1 and 2 : nR). In the experiment in which a part of the K^+ ions was substituted equimolarly for procaine (1 mmol/l Ringer) (Fig. 3) a much higher membrane hyperpolarization was recorded than that obtained with the same drug concentration but hypertonically added. Its amplitude (5.19 mV) was even higher than with 2.5 mM hypertonic procaine-Ringer. The recovery of the membrane potential in normal Ringer was however slower. In the control experiment (with 1 mM K^+ substituted for 1 mM Na^+) the hyperpolarization amplitude was of 8.72 mV (Fig. 4).

At an acid external pH (= 6) the procaine action on the membrane was different with respect to that at pH = 7.2. In 2.5 mM procaine-Ringer, the membrane was at the beginning slightly depolarized, and only after a recovery was it hyperpolarized. The amplitude of the hyperpolarization was about 1.43 mV (Fig. 5), hence evidently smaller than that in an alkaline medium. Bringing back the fibers in a procaine-free Ringer, the usual recovery of the membrane potential was observed (Fig. 5 : nR). A different effect was found, however, in experiments in which procaine was used in underanesthetic concentrations (1 and 0.5 mM). At 1 mM procaine concentration the membrane potential was at first slightly diminished but this depolarization (about 8.76 mV) was subsequently maintained constant during the whole experimental period (Fig. 6). When 0.5 mM procaine-Ringer was used, the same phenomenon took place, but the amplitude of the depolarization was only of 2.8 m V (Fig. 7).

DISCUSSIONS

As shown in an earlier work [1], the lipid leaflet of the outer membrane layer has prevalent cationitic exchange properties. The main active phospholipid, phosphatidyl-ethanolamine (PE), is organized in the resting state as laminar micellae, its molecules being in the "p_{in}" conformation when Ca^{2+} ions are bound in the structure. Kreutz [10] shows that PE is able to bind Ca^{2+} ions both in an alkaline and an acid medium, remaining in the same supermolecular organization (laminar micellae). The only change imposed by an acid pH is the modification of the molecular conformation from "p_{in}" to "p_{ex}". This change has however no important consequential effects on ion exchange, permeability and electrical properties of the membrane. These facts are considered by us as providing a good explanation for the data reported in literature [11] concerning the lack of any important modifications of the resting and action membrane potential at a limited modification in the external pH.

With respect to the membrane hyperpolarization by procaine, the data indicating a marked excitation threshold rise [9] and the fact that the anesthetic concentrations did affect neither the activity of the Na^+ and K^+ pump [2], nor the passive electrical properties [9], [21], conducted to the conclusion that the hyperpolarization was not implied in the blocking action and that this would occur only after the stimulus application [9], [20]. However, it is absolutely clear that any hyperpolarization results in a rise of the threshold depolarization. The experimental observations of Inoue and Frank [9] allowed us to estimate that the contribution of the hyperpolarization by procaine (about 1 mM) to the threshold rise was about 15 per cent, and consequently not totally insignificant. From this it also follows that the main component of the total blocking effect is not hyperpolarization, but another action, which does not modify the electrical charge of the resting membrane. Although there exist a lot of data concerning the procaine competition with Ca^{2+} [15] and the procaine interaction with K^+ [2], [3], [16], conclusions afforded by their authors are contradictory.

The mechanism of the procaine hyperpolarization would be, in our opinion, a phase transition from the K^+ globular PE micellae in the outer membrane layer, to the laminar ones, structured by Ca^{2+}. Such a mechanism would result in a small rise of the electrical membrane resistance and indeed, some authors have reported such an effect of procaine [7] [19]. Our experiments substituting (equimolarly) the external K^+ for procaine (Figs. 3 and 4) showed that procaine was able to remove K^+ from the PE globular micellae of the lipid leaflet belonging to the outer membrane layer, thus competing with the external Ca^{2+} for K^+ loci. In this way, a phase transition of these micellae took place, and therefore, a re-organization of the phospholipids in the laminar micellae with cationitic exchange properties. The direct effects of this phase transition were a small decrease in the ionic passive conductance and a hyperpolarization of the membrane. The phenomenon is produced, in all probability, by the cationic procaine, present in a great amount at pH = 7.2 [2]. The penetration of the cationic procaine into the outer membrane layer with cationitic exchange properties is evidently possible, but it is not possible in the inner membrane layer. The hyperpolarization by procaine, contributing in this manner to the threshold rise, represents one of the components of the complex blocking effect. A much more important component of this effect is a phenomenon produced also in the resting state, but which leaves the electrical charge of the membrane unchanged. It is well known that the onset speed of the blocking effect at a given procaine concentration increases with the rise of external pH [15]. At the same time, the penetration of the neutral form of the anesthetic into the inner layer is fully secured by its properties (lack of the electrical charge, high lipophilia).

On the basis of the "2-M.S.I." theory we are able to explain the excitation threshold rise, in full agreement with the strong inhibition of Na^+ active conductance rise (after the stimulus application), mainly as a stabilization phenomenon (actually a "superstabilization") of the inner membrane layer in the resting state. The effect on Na^+ active conductance led us to assume a procaine action localized in zones of

the lipid inner leaflet, with anionitic exchange properties which are, in the resting state, inaccessible to the cations. A rise over the normal value of the lyophobic sols stability in these zones does not induce a change of the membrane resting potential but determines a rise of the required Na^+ concentration to start the spike after the stimulus application. But this effect of procaine is not responsible for the inhibition of the K^+ active conductance rise.

At an acid external pH (= 6) the procaine cannot accomplish its blocking action, but its cationic form is able to penetrate into the outer membrane layer. Our present experiments demonstrated, on the one hand, that neutral procaine (free base) is mainly responsible for its blocking effect, and on the other hand, that the membrane hyperpolarization is due to cationic procaine. Thus, at an external pH = 6 we found that in a 2.5 mM procaine-Ringer solution the membrane was hyperpolarized, although less than at an external pH = 7.2 (1.43 mV against 4.69 mV). This hyperpolarization occurred after a previous small depolarization and, in addition, in a 1 mM procaine-Ringer solution, the membrane depolarization was the only effect observed. Evidently, one cannot confound this "acid depolarization" with the well-known lytic depolarization produced by procaine at high concentrations and at a normal pH [9], [15]. However, one may consider that the procaine depolarization effect, reported in some earlier papers [8], is a real effect (although most authors doubt it) and corresponds to the "acid depolarization" found by us. At an acid external pH and at a low concentration of procaine (2.5 mM and 1 mM respectively) we observed two different effects. One of these (the hyperpolarization) seemed to be determined by the higher concentrations of the drug, and the other (the depolarization), by its lower concentrations. Such opposite effects have been also observed with other anesthetics by Posternak and Arnold [13]. On this basis, Shanes [17] assumed that the stabilization effect could be determined by two opposite actions of the drug.

In connection with this conclusion, we should emphasize that in the case of procaine, the main component of blocking effect (the stabilization) [1] does not occur at pH = 6. On the other hand, our experiment conducted in a 0.5 mM procaine-Ringer solution (Fig. 7) showed that the "acid depolarization" was indeed a concentration-dependent phenomenon. The depolarization under these conditions was much smaller (2.8 mV) than in a 1 mM procaine-Ringer solution (8.76 mV). It is also obvious that the threshold of the hyperpolarization is higher than that of the depolarization, and that the latter has a greater onset speed. For these reasons we consider that the "acid hyperpolarization" having the same mechanism as the hyperpolarization in the alkaline medium, is partially reduced (masked) by the "acid depolarization".

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CHANGES IN CARBOHYDRATE AND FAT METABOLISM IN THE HAMSTER *(CRICETUS CRICETUS)* ACCLIMATED TO COLD

BY

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The liver and muscle glycogen content, the glucose phosphorylating capacity and the total glycolytic capacity of the liver in the hamster have been determined. The experimental results show that glycogen content of the liver and hind leg muscle is significantly lower and that glucose phosphorylating capacity is depressed in cold-acclimated hamsters. At the same time an increase of lactate formation was observed.
Exposure to cold induces an increase in the serum free fatty acid level and the liver total lipids.

Biochemical mechanisms that may account for the capacity of cold-acclimated and hibernating mammals to ensure the energy responsible for the maintenance of viability and functional integrity of tissues are only partially known. One might suppose that, functioning at low temperatures, certain tissues of these mammals may have undergone a phylogenetic adaptation of enzyme systems so that they are able to realize a different metabolic pattern as compared to nonhibernating mammals [13]. The extent to which one or another metabolic pathway is affected by prolonged cold exposure is still somewhat controversial.

Therefore we initiated, as a preliminary report, the assay of some biochemical parameters connected with carbohydrate and fat metabolism in cold-acclimated hamsters (*Cricetus cricetus*). We determined the liver and muscle glycogen content, the serum free fatty acids and the liver total lipids; then we carried out quantitative studies on the liver capacity to phosphorylate glucose and to convert it into lactate.

MATERIALS AND METHODS

Experiments were carried out on hamster males (*Cricetus cricetus*) grouped as follows: control animals maintained at room temperature (24 ± 2°C); active animals acclimated for 40 days to cold (6 ± 2°C). Experimental hamsters were caged individually and were fed with standard diet, carrots and water ad libitum. To determine the glycogen content, we measured the glucose formed after total hydrolysis in H_2SO_4 — 1 N of the liver and hind leg muscle samples, removed as quickly as possible.

Total glucose phosphorylating capacity was assayed from liver homogenate prepared with a Potter-Elvehjem homogenizer (with teflon pestle) in approx. 100 ml of medium, with a final concentration (mM): KCl = 100; TRIS = 50; EDTA = 6; $MgCl_2$ = 6; pH = 7.4 (adjusted with 20% KOH). The supernatant fraction obtained after centrifugation at 25,000 × g for 30 min. at 2°C was used and the assay was that described by Walker [22].

The maximal glycolytic capacity of the liver was determined from the same homogenate, incubated in a final volume of 5 ml (final concentration, mM): TEAM = 50; $MgSO_4$ = 8; ATP (Na salt) = 4; NAD^+ = 0.5; glucose = 20; K_2SO_4 = 15; and hexokinase (yeast, type V) = 1.0 U, pH = 7.4. From the mixture incubated at 37°C, samples (1.0 ml) were collected for 1 hour at 20 min. interval each and treated with 1 ml of $HClO_4$ 6%(w/v). After centrifugation and neutralization (with 20% KOH) lactate formation was assayed enzymatically [10]. The results were expressed in terms of protein content, assayed by the biuret method.

Estimations of the serum free fatty acids were made according to the procedure described by Novak [15] and the liver total lipids were determined gravimetrically after repeated extraction with a chloroform-methanol mixture (2 : 1).

Statistical evaluation of the data was accomplished by calculating the mean and the standard error of the mean for each parameter studied. Comparison of means was made by Student's t-test and all differences having a probability level of 0.05 or less were considered significant.

RESULTS AND DISCUSSIONS

The results of our determinations appear in table 1.

Table 1

Some biochemical parameters in hamsters aclimated to cold

Parameters	Tissue	Control animals (24 ± 2°C)	Cold-acclimated animals (6 ± 2°C)	± %
Glycogen mg glucose/g	hind leg muscle	8.7 ±0.44 (6)	3.3 ±0.24 (6) $p<0.001$	−62%
	liver	66.6 ±4.12 (7)	21.3 ±3.53 (6) $p<0.001$	−68%
Glucose phosphorylation (u/100 mg p/min.)	liver	0.56±0.03 (10)	0.12±0.01 (10) $p<0.001$	−78%
Lactate formation (µmoles/100 mg p/h)	liver	10.70±0.41 (10)	12.80±0.34 (10) $p<0.01$	+19%
Free fatty acids (mEq/l)	serum	1.76±0.06 (7)	5.08±0.18 (6) $p<0.001$	+65%
Total lipids (mg/g)	liver	51.77±2.08 (7)	59.75±1.75 (7) $p<0.02$	+15%

Note. In parantheses — the number of animals.

A prolonged exposure to cold is necessary to induce hibernation in hamster. In contrast to other hibernators, they have periodical arousals during which they feed. This fact besides other factors, such as the age of the animals, the length of the period to cold exposure, the season of the year when the experiment is carried out as well as the analytical procedures used, explains why literature data concerning the metabolism of hibernating animals, including the hamsters, are still somewhat controversial. According to some authors [17], [24] the liver glycogen content is higher during cold acclimation and hibernation, but is lower according to others [7] [12] [18]. The decrease of the tissue glycogen levels, together with the decrease of glucose phosphorylating activity and the increase of glycolytic capacity, reported by us and other authors in hibernators

[1] [4] [7] [16] [21] seems to indicate that glycogenolysis proceeds during cold exposure and that lactate formation results less from glucose and more from glycogen. In other words, the increased breakdown of glycogen in cold-acclimated hamsters rule out the possibility of a limitation, by the glucose phosphorylating system, of the reaction velocities leading to lactate formation. The decreased activity of liver phosphorylating system reported here does not agree with the results of Hannon and Vaughan [8] [9] who found out its rise. In fact these differences may reflect different cold exposure periods, which evidently influence the velocity of metabolic processes.

It seems possible to conclude that the decrease of the glucose phosphorylating activity ensures a "sparing" effect of glucose so necessary for the function of the heart and brain at low temperature and/or hypoxia.

Serum free fatty acids, whose concentration increases in cold-acclimated animals, become a principal source of energy after the inhibition of the hexokinase activity. The place of the lipid hydrolysis might be the adipose tissue [6] [11] [19] or the muscles [20]. This is accounted for by our results and those of other authors showing that the level of triglycerides from liver of animals exposed to cold increases or does not change [3].

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CALORIC VALUES OF THE BODY IN *MUS MUSCULUS SPICILEGUS* (PETÉNYI, 1881) AND *MICROTUS ARVALIS* (PALLAS, 1779)

BY

DOMNICA TÂCU

Studies were conducted on the effect of season (spring, summer, autumn) on the caloric values in *Mus musculus spicilegus* Pet. and *Microtus arvalis* Pall. The results demonstrate that the seasonal differences are not statistically significant. In *Mus musculus spicilegus* there are no statistically significant sex differences during the summer. Similarly, a comparison of the two species during the summer shows that there are no significant differences.

The problems related to the caloric values of the body of small mammals are highly important for the determination of ecosystem productivity but, nevertheless, few data on this subject are found in literature [2] — [6].

The present paper deals with the caloric values of the body of *Mus musculus spicilegus* (Petényi, 1881) and *Microtus arvalis* (Pallas, 1779), frequently occurring in the crops of Romania.

MATERIAL AND METHODS

Mus musculus spicilegus individuals were collected from the alfalfa and corn fields of the Research Institute for Cereals and Technical Plants Fundulea, located in the surroundings of Bucharest (26°32′E, 44°28′N), and *Microtus arvalis* specimens were captured in the clover fields of the Central Research Station for Grassland, Brașov — Măgurele (25°33′E, 45°39′N). On the whole 169 determinations were conducted on 43 animals.

The animals captured in traps were weighed, opened along the ventral line and dried for 3 days in a lyophilizing apparatus. The dried animals were weighed and ground in a grinding apparatus. The resulting material was then divided into samples of 1 — 1.2 g each, and wrapped in paper instead of being pressed into tablets, so as to avoid the loss of fats due to compression. The combustion proper was carried out according to the method specified in the official standard for the evaluation of caloric values of solid fuels. A Berthelot calorimeter was used. The ash was weighed after combustion.

Caloric values were determined for dry matter (Wg), ash-free dry matter (Wg_{A-F}), and in addition, the water and ash contents in the dry matter. The seasonal variation of these parameters was investigated for two years and the two species were compared in the seasons under study. In *Mus musculus spicilegus* the values determined in summer were compared in terms of sex. The caloric values were measured in cal/g. The statistical data evaluation was made according to the dispersional analysis method.

RESULTS

Significant seasonal differences were recorded in *Mus musculus spicilegus* as to the fresh weight (Table 1), with peak values during summer

($d^{*} = 5.44^{++}$; LD 1** = 1.01), and to the ash content, with peak values during autumn ($d = 1.16^{++}$; LD 1 = 1.01). No significant seasonal variations resulted for the other parameters investigated.

Table 1

Caloric values in *Mus musculus spicilegus* (Petényi, 1881) determined for two years

Season	Spring	Summer	Autumn
	n=7	n=14	n=14
Fresh body weight (g)	14.75±1.83	15.56±2.55	12.12±2.20
Water in the whole body mass (%)	62.83±2.56	63.54±3.86	63.15±4.35
Ash in the dry matter (%)	7.92±0.66	8.84±1.09	10.02±1.34
Caloric values of dry matter Wg (cal/g)	6607±334	6297±372	6039±359
Caloric values of ash-free dry matter (Wg_{A-F}) (cal/g)	7175±334	6906±366	6712±372

Parameter variations during summer in terms of sex are given in table 2; but no significant differences were obtained for any of them.

The results for *Microtus arvalis* are listed in table 3. No significant seasonal variations of the parameters could be established in this species (low numbers of cases considered).

A comparaison of the two species (*Mus musculus spicilegus* and *Microtus arvalis*) during the summer season showed that there were no significant differences between most of the parameters studied, except for the fresh weight, which attained peak values in *Microtus arvalis* ($d = 19.59^{++}$; LD 5 = 17.8).

Table 2

Caloric values in *Mus musculus spicilegus* (Petényi, 1881) determined in summer

Sex	♂♂	♀♀
	n = 7	n = 7
Fresh body weight (g)	15.40±1.68	15.25±3.80
Water (%) in the whole body mass	64.39±4.24	63.49±2.64
Ash (%) in the dry body weight	8.81±1.02	9.37±1.00
Caloric values of dry matter (cal/g)	6123±367	6355±300
Caloric values of ash-free dry matter (cal/g)	6712±357	7010±283

* d = difference.
** LD = limit difference.

During autumn, in most cases the differences between these species were not significant, except for the fresh weight, for which peak values were recorded in *Microtus arvalis* ($d = 9.41^{++}$; LD 1 = 7.5).

Table 3

Caloric values in *Microtus arvalis* (Pallas, 1779)

Season	Summer	Autumn
	n = 4	n = 4
Fresh body weight (g)	37.87±10.25	21.52±4.57
Water (%) in the whole body mass	61.78±2.48	59.24±5.69
Ash (%) in the dry body weight	8.27±0.72	9.32±2.38
Caloric values of dry matter (cal/g)	6309±70	6233±729
Caloric values of ash-free dry matter (cal/g)	6878±124	6861±642

DISCUSSION

The data in the present paper for *Mus musculus spicilegus* and *Microtus arvalis* are different from those mentioned in literature.

Lower figures of the caloric values of dry matter (Wg) and of the caloric values of ash-free dry matter (Wg_{A-F}) were obtained by Gorecki [4] in a number of shrews and rodents, by Myrcha [6] in shrews and by Hansson and Grodzinski [5] in *Microtus agrestis* L. These probably arise from the fact that a different technique of calorimetric combustion was used. However, the values obtained by them for water and ash contents in the dry matter (in per cent) were higher. This may probably be due to the fact that in the present study the material was been partially dried by lyophilization.

The present data for Wg and $Wg_{(A-F)}$ are in agreement with those obtained by Bergeron [1] in small mammals.

There is no significant seasonal variation of the caloric values of dry matter and of the caloric values of ash-free dry matter from spring to summer and autumn.

The hypothesis that the parameters studied are not significantly influenced by the sex [6] is confirmed by the observations on *Mus musculus spicilegus*.

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EFFECTS OF ALLOXAN DIABETES AND SUBDIAPHRAGMATIC VAGOTOMY ON HYPERPHAGIA AND OBESITY INDUCED BY MECHANICAL ISOLATION OF HYPOTHALAMIC CENTERS INVOLVED IN FEEDING BEHAVIOUR

BY

V. P. HEFCO and P. ROTINBERG

On the basis of data obtained in rats with subdiaphragmatic vagotomy or alloxan diabetes, it is concluded that hyperphagia and obesity induced by mechanical isolation of the medial hypothalamic area, VMH included, from the lateral hypothalamus, is determined primarilly by hyperinsulinemia.
It is suggested that insulin, besides a stimulatory effect on the satiety center, has a lower facilitatory action on the structures that determine eating.

As shown in a previous paper [13], the mechanical isolation of the satiety center from the feeding center results in hyperphagia and obesity, different from that induced by ventromedial hypothalamic (VMH) nucleus lesion. A partial list of factors that might influence the hypothalamic centers which control food intake comprises : the specific dynamic action of food (thermostatic hypothesis) [6] [30]; the availability and utilisation of glucose from body fluids (glucostatic hypothesis) [20]; concentration of circulating fat metabolites (lipostatic hypothesis) [16]; concentration of serum amino acids [22]; sensations from the digestive tract [15] [27]; signals from higher segments of the central nervous system [1] [19] [23]; palatability and aroma of food substances [7].

Mechanical isolation of the VMH from lateral hypothalamus (LH) causes a rise in insulinemia without affecting blood sugar level [14] or aminoacidemia (unpublished data), as well as an increase of NEFA and serum triglycerides [12]. Under our experimental conditions no causality relation was found between aminoacidemia and food intake (personal data). The experiment reported below aim at establishing the role of the vagus nerve and of insulin in the determination of hyperphagia and obesity induced by the isolation of the satiety center from the hunger center. Our results point out that insulin plays one of the main roles in determining this type of hyperphagia and obesity.

MATERIAL AND METHODS

Male Wistar rats, maintained at 22°C and McCollum standard diet, were used. Hypothalamic isolation was carried out according to the stereotaxic method of Halász and Pupp [11], with some differences indicated previously [13]. Histological control of the brain isolation was performed according to Guzman-Flores et al. [10]. Subdiaphragmatic vagotomy was per-

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formed according to Snowdon and Epstein [29], 1.5 month after hypothalamic isolation. Alloxan diabetes was induced by i.v. injection of 45 mg recrystallized alloxan/kg b.w. after a previous 24 — 28-hour starvation. For the next 3 days 4 U/kg/day insulin (Biofarm) were administered subcutaneously. The degree of diabetes was established by assaying glycemia, determined by the method of Asatoor and King [3]. In view of determining food intake, rats were kept in Ruffeger-type metabolic cages.

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

RESULTS

1. *Effect of vagotomy on food intake and body weight.* Hyperphagia caused by mechanical isolation of the medial hypothalamus disappears following subdiaphragmatic vagotomy (Fig. 1). The degree of obesity

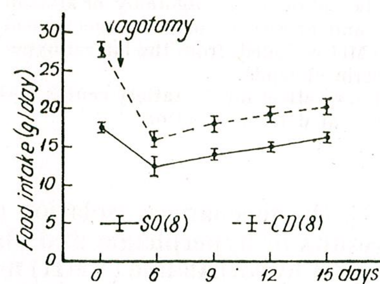

Fig. 1. — Effect of subdiaphragmatic vagotomy on hyperphagia induced by mechanical isolation of the medial hypothalamus.

CD = rats with complete isolated medial hypothalamus; SO = sham-operated rats. Values are M±SE. Number of rats is given in brackets.

of hyperphagic rats is not modified 20 days after vagotomy. At the same interval the weight of sham-operated (SO) rats decreases unsignificantly. A hundred and fifteen days after vagotomy, the weight of obese rats decreases significantly while the weight of SO rats increases unsignificantly (Table 1).

Table 1

Changes of body weight (g) in rats with isolated medial hypothalamus (CD) after subdiaphragmatic vagotomy or alloxanization SO=sham-operated rats. Values are M±SE. The number of rats is given in brackets.

Groups	Vagotomy			Alloxanization		
	Prevagotomy	Postvagotomy		Prediabetic	Postdiabetic	Glycemia mg%
	1.5 month	20 days	115 days	1.5 month	45 days	45 days
SO	310±5.4 (8)	304±6 (8)	316±6.2 (8)	251±4.5 (7)	220±6.2 (7)	255±9.1 (7)
CD	398±7.2 (8)	398±8 (8)	355±8.4 (8)	303±5.8 (7)	215±7.4 (7)	265±8.0 (7)

2. *Effect of alloxan diabetes on food intake and body weight.* In rats with intact nervous connections between the hypothalamic centers involved in food intake, the destruction of beta-pancreatic cells determines obvious long-lasting hyperphagia, associated to a decrease of body weight (Fig. 2 and Table 1). Hyperphagic rats after alloxanization reduce food intake

even under the level of that previous to the intervention on the hypothalamus (Fig. 2). Body weight also decreases very much (Table 1).

DISCUSSION

Our data show that vagotomy or alloxanization abolish hyperphagia and reduce obesity induced by mechanical isolation of the medial hypothalamus, VMH included, from the lateral hypothalamic (LH) area.

The influence of food intake by the vagus nerve cannot be assigned to afferent signals, starting at the level of the gastric strech receptors described by Paintal [25] or of hepatic glucoreceptors [24] as these signals stimulate VMH nucleus. The abolition of hyperphagia might be due to the impossibility of the efferent transmission through the vagus nerve. In its turn, this might affect the motility of the digestive tract, its secretion

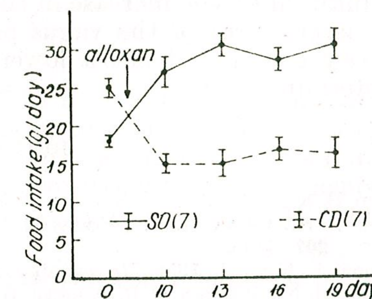

Fig. 2. — Effect of alloxan diabetes on food intake in hyperphagic rats.
Legend as in fig. 1.

or that of annex glands or of the endocrine pancreas and/or might act directly on some metabolic processes, which could modify the value of signals acting on hypothalamic centers implied in food control. The mechanical isolation of hypothalamic centers results in an increase of the parasympathetic tonus of the visceral area of the vagus nerve [13], which might determine an increased motility of the digestive tract, reducing the value of satiety signals; it might determine a gastric hypersecretion, responsible for the hyperphagia of CD rats, as well as in the case of rats with lesions of the VMH nucleus [28].

A comparison of results obtained in food intake following vagotomy and alloxanization shows that under the conditions of the mechanical isolation of hypothalamic nuclei implied in the control of food intake, the main cause of hyperphagia is hyperinsulinemia, occurred as a result of the increase in the tonus of the hypothalamic parasympathetic area, after removing the inhibitory influences exerted by the medial hypothalamic area. The lateral hypothalamic area influences the vagus nerve by its connections with the motor nucleus of this nerve [21]. The stimulation of the vagus nerve results in insulin hypersecretion [9]. Indeed, insulin administered exogenously is capable of eliciting feeding in a satiated animal [5] and its chronic administration determines hyperphagia and obesity [18]. The involvement of the vagus nerve in the control of feeding and body weight by its influence on insulin secretion also resulted from stimulation experiments of the dorsal motor nucleus of the vagus [17] or by direct vagal stimulation [26] which induced eating in satiated animals.

As shown by our data, if we assign to hyperinsulinemia a causal role in hyperphagia and obesity, as recorded in CD rats with isolated

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medial hypothalamus, we must admit that either insulin, or metabolic signals induced by it, act both on the satiety center [2] [4] [8] and on the lateral hypothalamic area or other nervous structures that initiate feeding. The stimulating effect on VMH is stronger than on the latter formations. Indeed, if insulin stimulated only VMH nucleus [2] [4] [8], which in its turn would inhibit the feeding center from the LH, then in alloxanized CD rats, food intake would not decrease. The behaviour of CD rats after vagotomy might point to reduced insulinemia. In normal rats vagotomy lowers portal venous insulin secretion [9].

In conclusion, without denying the role of other signals in the control of eating behaviour, our results point out that hyperphagia and obesity, occurring after the mechanical isolation of the hypothalamic medial area from the LH area, might be determined primarily by hyperinsulinemia, induced by the increase in the tonus of the parasympathetic system in the visceral area of the vagus nerve. It seems that insulin stimulates the satiety center and has a lower facilitating action on the structures that determine eating.

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L'INFLUENCE DU CHAMP ÉLECTROMAGNÉTIQUE SUR LA CHOLESTÉROLÉMIE

PAR

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Guinea-pig treatment by pulsating low intensity EMF results in a decreased concentration of total, free and serum-esterified cholesterol. This decrease is more pronounced after 10 days of treatment. The decrease in total cholesterol is due to free cholesterol, after 5 days, and to both free and esterified cholesterol, after 10 days.

Le champ électromagnétique (CÉM) exerce une influence complexe sur l'organisme, affectant les fonctions et le métabolisme des différents organes ou tissus. Chez les animaux soumis au traitement par du CÉM pulsant on connaît des changements concernant certains aspects du métabolisme glucidique [1], [2], [3], [4], protéique [5], [6], lipidique [7], [8], etc.

L'approfondissement des connaissances concernant la réponse de l'organisme à l'action du CÉM suppose aussi l'investigation de ses effets sur d'autres aspects métaboliques. Dans l'un de nos travaux antérieurs [9] nous avons exposé les résultats obtenus concernant l'action du CÉM pulsant, de faible intensité, avec interruption, sur la dynamique des triglycérides et des acides gras libres du sang.

Nous présentons dans cette Note les résultats de l'action du CÉM, avec les mêmes caractéristiques physiques, sur le taux du cholestérol total, du cholestérol libre et de celui estérifié du sérum du cobaye.

MATÉRIEL ET MÉTHODES

Nos recherches ont été effectuées sur des cobayes de même taille et des deux sexes, maintenus dans des conditions identiques. Les animaux ont été nourris pendant l'hiver avec des betteraves, du foin et de l'avoine et pendant le printemps avec de l'avoine et du fourrage vert. Pour une période de 5 jours et aussi de 10 jours, à intervalles de 24 heures et à la même heure du matin, les animaux ont été soumis journellement 5 minutes à un CÉM pulsant de 180 Öe, appliqué avec interruption d'une seconde pour trois secondes d'exposition [10].

Le taux du cholestérol total et du cholestérol libre du sérum a été dosé par une microméthode avec du Ferrum sesquichloratum [11] modifiée et adaptée par l'un de nous (à publier). Le cholestérol estérifié a été calculé par la différence entre le cholestérol total et celui libre. Les échantillons de sang à analyser ont été récoltés des vaisseaux cervicaux, les animaux étant décapités après 12 — 14 heures d'inanition, toujours à 9 heures du matin, c'est-à-dire aussi après une heure à partir de leur dernière séance de traitement par du CÉM.

Les résultats ont été interprétés statistiquement, en utilisant pour appréciation de la signification le test « t » de Student.

RÉSULTATS ET DISCUSSIONS

Le taux du cholestérol total, du cholestérol libre et de celui estérifié du sérum des cobayes témoins et de ceux traités par du CÉM 5 ou 10 jours est représenté dans la figure 1. Il résulte que la concentration du cholestérol total du sérum chez les animaux traités par du CÉM baisse après 5 jours, de même qu'après 10 jours de traitement, cette dernière étape étant plus accusée et statistiquement significative ($p < 0,002$). Chez les animaux soumis 5 jours à l'action du CÉM le cholestérol libre présente un abaissement semblable à celui du cholestérol total ; après 10 jours de traitement, le cholestérol libre du sérum de ces animaux diminue plus que la moitié de la valeur témoin. Les variations du cholestérol estérifié sont plus atténuées : après 5 jours de traitement la concentration du cholestérol estérifié est, pratiquement, sans modification, tandis qu'après 10 jours elle diminue de presque 20 %.

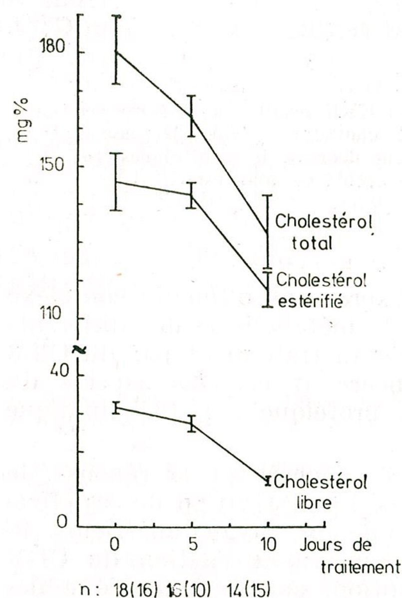

Fig. 1. — Le taux du cholestérol total, du cholestérol estérifié et de celui libre (en mg/100 ml sérum) après le traitement des cobayes par CÉM pendant 5 et 10 jours. O = témoins ; n, nombre des animaux utilisés au dosage du cholestérol total (les chiffres entre parenthèses indiquent le nombre des animaux utilisés au dosage du cholestérol libre et estérifié).

Nos données montrent que le traitement des cobayes par un CÉM pulsant de faible intensité détermine la baisse de la concentration du cholestérol total, du cholestérol libre et de celui estérifié du sérum, l'abaissement étant évident surtout si on continue l'action du CÉM pendant 10 jours. Les valeurs obtenues indiquent que la baisse du cholestérol total est due, après 5 jours de traitement au cholestérol libre, tandis qu'après 10 jours tant au cholestérol libre qu'à celui estérifié.

L'abaissement du cholestérol du sérum sous l'action du champ magnétique et d'ondes électromagnétiques a été constaté aussi dans les recherches effectuées par Pautrizel et coll. [8] chez les animaux avec hypercholestérolémie provoquée par un régime alimentaire renfermant 1 % de cholestérol.

Chez les cobayes traités à CÉM, on peut attribuer la diminution du cholestérol tant à l'intensification des processus de catabolisme qu'à l'inhibition de sa synthèse. Le foie détient le rôle principal dans le catabolisme de même que dans la biosynthèse. Le cholestérol peut être catabolisé dans le foie dans des acides biliaires, et, au niveau des glandes surrénales, des glandes sexuelles et du placenta, il peut être utilisé comme précurseur dans la synthèse des hormones stéroïdiques. La synthèse du cholestérol est réalisée dans toutes les cellules, mais seulement le foie,

l'intestin et la peau fournissent une quantité estimable de cholestérol dans le sang.

Chez les mammifères, le contrôle des processus impliqués dans le métabolisme du cholestérol se réalise par de nombreuses voies, dont les détails restent encore inconnus. Il est donc difficile de trouver une explication adéquate pour le mécanisme concret de l'action du CÉM sur le métabolisme du cholestérol. De plus, l'influence du CÉM sur le métabolisme du cholestérol de quelques organes, comme les glandes surrénales [12], ne produit pas les mêmes effets que dans le cas du cholestérol sanguin.

Le rapport entre le cholestérol sérique libre et le cholestérol estérifié est de 2,33 fois plus petit chez les animaux soumis au traitement par le CÉM pendant 10 jours, comparativement au même rapport chez les animaux normaux. C'est ce qui nous peut conduire à la possibilité d'une action du CÉM sur des facteurs qui règlent la synthèse hépatique du cholestérol. Pour élucider ces phénomènes de grande complexité, le problème qui se pose immédiatement est celui de poursuivre l'action du CÉM sur le cholestérol présent dans d'autres organes, surtout dans le foie.

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NEUTRON-INDUCED CHROMOSOMAL ABERRATIONS IN MEIOSIS AND THE BEHAVIOUR OF SEX CHROMOSOMES IN THE MALE RAT

BY

VERONICA STOIAN and M. D. NICU

The effect of small and medium doses of neutrons on meiosis in the male rat can be characterized by the occurrence of various types of chromosome aberrations : tetravalents, extrachromosomes, aneuploid cells and "apparently polyploid cells". Their frequency varies in relation to dose and recovery time. The behaviour of heterogametic sex chromosomes, which has proved to be similar to that of most mammals, is also discussed.

Two aspects have been approached in this paper : the effects caused by small and medium doses of neutrons on meiosis and the behaviour of the sex bivalent in the rat. Studies on meiosis in the mammals are still relatively few, just as those on the effects of neutrons. As for the second aspect here analysed, it is known that in mammals, in meiosis, the sex chromosomes have a different behaviour as compared with the autosomes. These differences become visible because of the cytological, structural and mechanical properties of the sex chromosomes. Such studies have already been reported on mice, hamsters and men [3] [7] [11], and since our cytological preparations were fairly good, we endeavoured to carry out such a study on rats, too.

MATERIAL AND METHODS

For the study of the heterogametic sex meiosis and the effect of fast neutrons on the meiotic chromosomes, rats of the Sprague-Dowley line were used. A batch of 12 male animals were irradiated with fast neutrons of 5 KeV — 10 MeV and doses of 100 R and 200 R. The animals were killed after various periods of recovery (1 day, 3, 7 and 30 days). Another two animals were used as control. The meiotic preparations were made according to Meredith's [9] and Luciani et al.'s [8] technique, which was adapted to suit our own material.

About 200 meiotic cells were examined for each sample and the chromosome aberrations caused by the neutrons employed were recorded. The behaviour of the sex chromosomes was registered on preparations and photographs, especially in meiotic prophase I and metaphase I, and the possible chromosome aberrations induced by the neutrons were identified in metaphase I and II and their frequency was calculated in per cent.

RESULTS AND DISCUSSIONS

a) Chromosome aberrations induced by neutrons, 100 R and 200 R

Table 1 contains the types of chromosome aberrations observed and their frequency. Since the study of chromosome aberrations in meiosis

is rather difficult, we tried to make their detection more certain by following up only a few types of aberrations : tetravalents, extrachromosomes, aneuploid cells and "apparently polyploid cells".

Table 1

The type and frequency of the chromosome aberrations induced by neutrons (100 R and 200 R) in the meiosis of the rat

Dose (rads)	Time of recoversy (days)	Number of cells analyzed	Metaphase I							Metaphase II		
			Number of metaphases I analyzed	Normal metaphases I (%)	Abnormal metaphases with IV		With extra-chromosomes	Apparently polyploid cells	Abnormal metaphases I analyzed (%)	Number of metaphases II analyzed	Normal metaphases II (%)	Apparently polyploid cells (%)
					1	2						
Control	—	219	189	96.3	—	—	0.6	3.1	—	30	98.2	1.8
100 R	1	200	168	77.9	3.5	1.7	0.9	16.0	22.1	32	100	—
	3	210	174	84.1	1.1	1.7	2.2	10.9	15.9	36	94.5	5.5
	7	202	186	78.2	2.6	0.5	1.0	17.7	21.8	16	93.8	6.3
	30	211	170	87.8	1.1	—	2.9	8.2	12.2	41	97.6	2.4
200 R	1	188	169	77.2	1.7	2.3	0.5	18.3	22.8	19	94.8	5.2
	3	211	187	78.2	—	0.5	2.6	18.7	21.8	24	95.9	4.1
	7	252	231	86.3	3.4	1.2	0.4	7.7	12.7	21	95.8	4.2
	30	254	213	86.3	2.4	0.5	0.5	9.3	12.7	41	97.6	2.4

The frequency of tetravalents (IV) observed in metaphase I is not high : it ranges from 0.5 to 3.5 per cent. However, it is interesting to note a drop in their frequency depending on their recovery period and this becomes even more visible 7 days after recovery. Similar data were observed on rat embryos exposed to X-rays (300 — 600 R), where the number of abnormal metaphases fell after 7 or 8 days [12]. One can also see in the table a higher frequency of the cells with tetravalents in the animals irradiated with 200 R. For instance, after 30 days the number of cells with tetravalents for the 100 R dose is 1.1 per cent and zero (respectively, cells with 1 IV and cells with 2 IV), and for the 200 R dose it is 2.4 % and 0.5 % (Plate I, *a*).

The extrachromosomes or unidentified chromosomes or small centric chromosomes (Plate I, *c*) are sporadically observed both in the treated material and in the control. The reason for which their frequency in the irradiated material does not seem to depend either on the dose or on the recovery time is, we think, the fact that they can also be found in the control. Their significance is not elucidated either in meiosis or in mitosis. The extrachromosomes can be observed in all the stages of the meiosis without apparently being associated with any other chromosomes [6]. This non-association with other chromosomes seems to be accounted for by the fact that they consist mainly of centrometric heterochromatin and it is almost impossible for chiasmata to be formed in such areas.

The frequency of the polyploid germ cells (tetraploid, octoploid and aneuploid cells) is very high. Such polyploid cells also occur

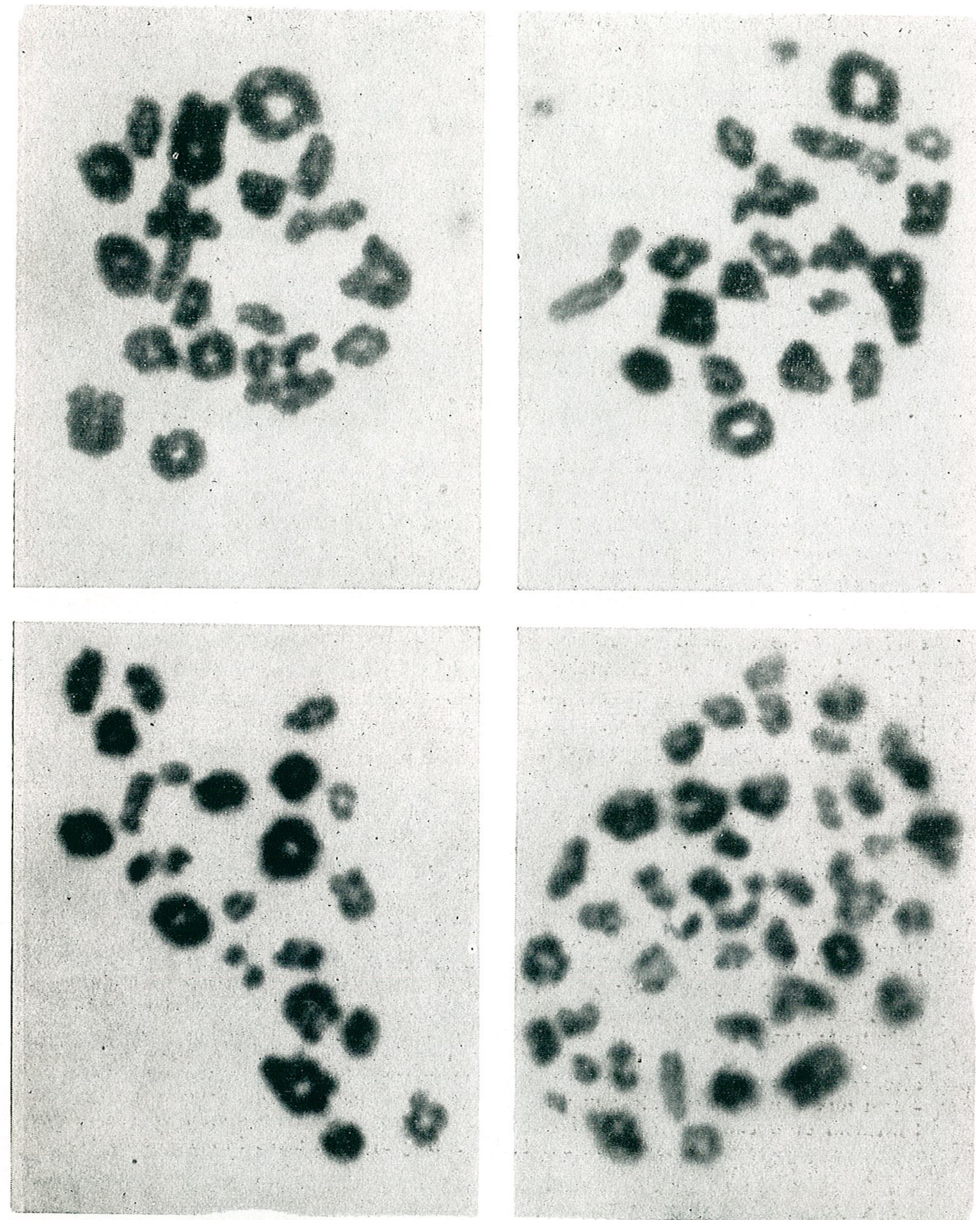

PLATE I. — Chromosomal aberrations induced by neutrons in meiosis in rat : *a*, metaphase I with 19 bivalents and one tetravalent ; *b*, metaphase I with one simple break between the X and Y chromosomes ; *c*, metaphase I with extrachromosomes ; *d*, tetraploid cell in metaphase I with 42 bivalents having the contour like a single cell.

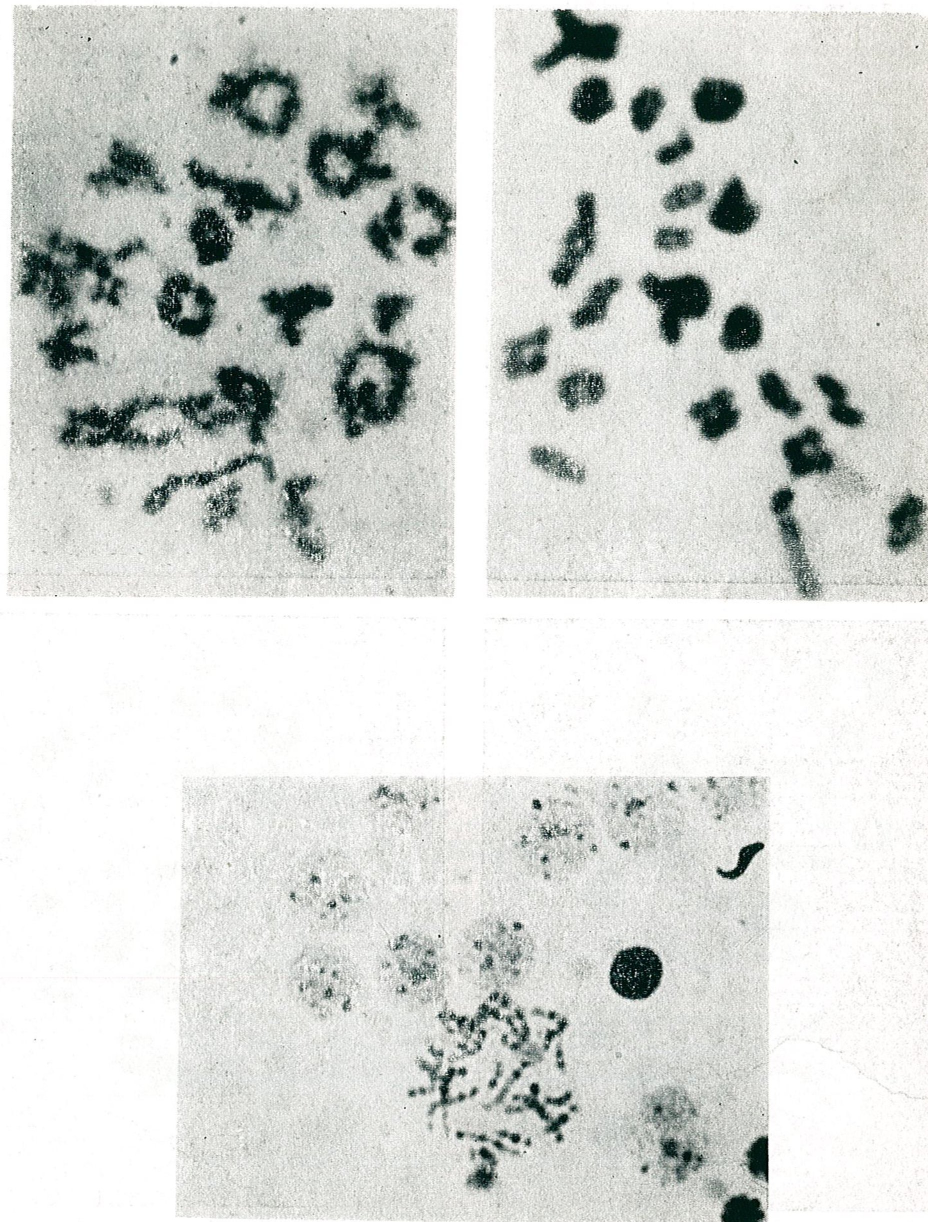

PLATE II. — The behaviour of the sex chromosomes and the configuration of the sex vesicle in the male rat: *a* and *b*, the association of the sex chromosomes is of the end-to-end type in diakinesis and metaphase I; *c*, the configuration of the sex vesicle in pachytene.

in the control and have a smaller frequency (for instance 3.1 per cent) as compared with the irradiated material where they reach even 18.3 per cent for the 200 R dose one day after irradiation.

In the same table, one can see that 30 days after recovery the proportion of apparently polyploid cells falls to 8.2 per cent and respectively 9.3 per cent (for 100 R and 200 R) in metaphase I, and in metaphase II their frequency is even lower (2.4 per cent).

Indeed, it seems that as compared with the X-rays, for instance, the neutrons induce a high frequency of polyploid cells in mitosis [5]. However, the polyploid germ cells are still a puzzle; that is why they are called "apparently polyploid cells" (Plate I, *d*).

Which is the explanation? Since the animal cell membranes are destroyed during the preparation of the chromosomes for a good display, it is impossible for us to know whether these cells represent true polyploid cells or only perfectly synchronised adjacent cells. The second supposition suggests that the last two maturation divisions of spermatogonia and the meiosis for two daughter cells often occur in absolute synchronism [13]. On the other hand, electron microscopy [2] [4] reveals intercellular bridges between the germ cells, so that the adjacent cells may appear as polyploid cells with chromosomes in the same degree of contraction. For a certain degree of certainty in the detection of the apparently polyploid cells in meiosis, these must contain chromosomes in exactly the same degree of contraction, and their setting must give the impression of a single cell, which must contain tetravalents. We followed as close as possible these criteria. The significance of these cells is not known and we must take very great care when we interpret them.

b) The behaviour of heterogametic sex chromosomes in rat

In order to interpret the observations made of the meiotic behaviour of the sex chromosomes in rat, the following criteria were considered: the associating time of the sex chromosomes during diakinesis; the way in which the sex chromosomes segregate; the configuration of the sex vesicle in pachytene.

The association of the sex chromosomes in the heterogametic sex of rats in diakinesis and metaphase I is of the end-to-end type (Plate II, *a*, *b*). In this type of association, the homologous segment is very small and the presence of a chiasma is still a matter of controversy. Köller assumed the existence of a partial synapsis and the formation of chiasma between the human sex chromosomes, but Eberle [3] refused to accept the existence of synapsis and chiasma.

Electron microscopic studies have not been able so far to elucidate the matter. Thus, Solari [11] identified side links in a common area of the X—Y pair and the development of the synaptinemal complex in that area, whose formation, however, is not sufficient for the existence of a chiasma.

Like in most species of mammals, in rat the sex chromosomes also separate in the prereductional type. This separation is preceded by the typical behaviour of the sex bivalent in the first meiotic prophase. By the prereductional separation of the sex bivalent during the first meiotic division two types of secondary spermatocytes appear, some with one X-chromosome, others with one Y-chromosome. During the second

meiotic division, the sex chromosomes split lengthwise into two halves identical in shape and size.

The configuration of the sex vesicle in rat is typical of the heterogametic sex chromosomes which are not homologous and which stick together by end-to-end association. Consequently, the pairing and formation of chiasma cannot take place. Instead of this, the sex chromosomes are prematurely condensed in relation to the autosomal bivalents and a sex vesicle is formed (Plate II, *c*). This early condensation prevents the crossing-over and maintain a normal balance for sex determination.

The species with end-to-end association have an ordinary sex vesicle, which represents the original sex chromosomes ; the sex vesicle is dissolved during the late pachytene or diplotene, and the sex pair is kept together by end-to-end association.

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KINETICS OF RADIOSTRONTIUM CONCENTRATION IN FISH FOR WATER UNIQUE AND CHRONIC CONTAMINATIONS

BY

ION CHIOSILĂ and ILCA MĂRGINEANU

This work presents the study of ^{85}Sr concentration kinetics in fish for water unique and chronic contaminations. The experimental data of the radiostrontium concentration, obtained on *Carassius auratus gibelio* and *Pseudorasbora parva*, were introduced into the equations suggested by a mathematical pattern of the radionuclide migration to a bicompartimental system. Therefore, a quantity of water, filtered by fish — $I \approx 0.5$ ml/g/day — was evaluated and the radioisotope was integrally retained from it. "I" — the time evolution of the radionuclide concentration ($\Lambda_{f\max}$, $t_{\max}$, CF) — can be foreseen.

Our paper presents an approach on ^{85}Sr concentration kinetics in fish for water unique and chronic contaminations. By introducing experimental data of radiostrontium concentration, obtained on *Carassius auratus gibelio* and *Pseudorasbora parva*, into equations suggested by a mathematical model of radionuclide migration in a bicompartmental system, there could be evaluated the quantity of water filtered by fish ($I \approx 0.5$ ml/g/day), of which the radioisotope was integrally retained. Knowing "I", we could prognosticate the development of radionuclide concentration ($\Lambda_{f\max}$, $t_{\max}$, C F).

The development of nuclear technology and continual increase of radioactivity in biosphere require a correct evaluation of contamination risks for different species including man. Therefore, knowledge of concentration kinetics of radionuclides is of great interest [1] — [7] [9] — [15]. However, several biological parameters conditioning accumulation and radioisotopes fixation in live organisms are not yet well known.

The present paper affords experimental data referring to ^{85}Sr concentration in fish for water unique and chronic contaminations and the analysis of these data on the basis of a mathematical model.

In our investigations, ^{85}Sr was used — gamma emitter with half-time (Tr) = 65 days — not ^{90}Sr-fission product (beta emitter with Tr=28.1 years), as the latter does not allow a good counting of the activity of the whole fish organism.

The contamination instances mentioned above occur either in the case of a nuclear accident (atomic explosion, deterioration of nuclear power plants and nuclear reactors, etc.), when a water unique and also acute contamination is produced, or in the case of some repeated radionuclide over-flowings from nuclear installations for water chronic contaminations. Knowledge of incorporation kinetics allows prognostication

of time dependence of radionuclide concentration in organism depending on the type of contamination and initial activity, offering the possibility to obtain significant biological parameters for fish metabolism.

EXPERIMENTAL MODEL

The experiments were performed on *Carassius auratus gibelio* and *Pseudorasbora parva* adults, weighing 25 — 30 g and respectively 5 — 7 g. The fish were placed, 5 samples each, in aquaria of 32 l and 10 l. Water contamination was performed with $^{85}SrCl_2$ (Amersham — England) at the beginning or during experiments, as follows:

a) for acute or unique contamination with 500 pCi/ml, we had in view radionuclide concentration as a function of time, and for other experiments with 50, 150, 250 and 500 pCi/ml we aimed at a concentration depending on water initial activity;

b) in the case of chronic contamination, we continually added radionuclide for preserving water contamination (about 500 pCi/ml).

Fish food, consisting in *Tubifex* sp., was not contaminated. We had in view only radiostrontium concentration in fish, directly in water, at the level of branchiae and scales.

Water and fish activity were measured in the "whole-body" system [2] [8] on a Philips monochannel spectrometer with an efficiency of 2.4 %.

The significance of the differences between the averages of fish specific activities for the two kinds of contaminations and also the control of the probability that the dispersion of the results will be equal, were tested by means of "t" (Student) and "F" (Snedecor) tests in a calculation program.

MATHEMATICAL MODEL OF FISH ISOTOPES INCORPORATION

a) The case of water (acute) unique initial contamination. Considering the isotope migration phenomenon in the closed bicompartmental system [9] [10] [11] water — fish, where every compartment is supposed to be homogeneous compared to isotope distribution, the equation giving the specific activity in fish at "t" moment is:

$$\frac{d\Lambda_f(t)}{dt} = I \cdot \Lambda_w(t) - (\beta + \lambda)\Lambda_f \qquad (1)$$

where $\Lambda_f(t)$ = specific activity in fish at "t" moment (in pCi/g wet weight), $\Lambda_w(t)$ = water specific activity at "t" moment (pCi/ml), I = water quantity of which 1 g fish retains the whole radioisotope quantity/1 day (ml/g/day); $\beta = \frac{0.693}{T_b}$ = coefficient of biological turnover (day^{-1}); $\lambda = \frac{0.693}{T_r}$ = disintegration constant (day^{-1}); V_f and V_w represent fish, respectively water volume.

The theoretical curves of fish specific activity (Λ_f) and of concentration factor (CF) defined as $\Lambda_f(t)/\Lambda_w(t)$ are presented in figure 1 for the following arbitrary values of the parameters:

$$I = 1 \text{ ml/g/day},\ \beta = 6.3 \cdot 10^{-3} \text{ day}^{-1},\ \lambda = 10.7 \cdot 10^{-3} \text{ day}^{-1},$$

$$\frac{V_f}{V_w} = 5 \cdot 10^{-3}, \text{ and for water initial activity } \Lambda_w^0 = 1{,}000 \text{ pCi/ml}.$$

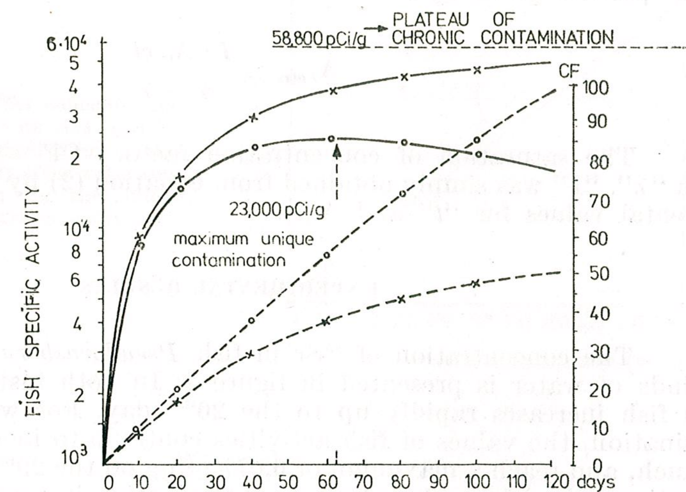

Fig. 1. — Dynamics of radiostrontium concentration by fish (——) and of concentration factor (– – –) in case of water radioactive unique (o) and chronic (×) contamination — theoretical curves.

The time value at which $\Lambda_f(t)$ reaches the maximum (t_{max}), obtained by derivation of equation (1), does not depend on the water initial activity Λ_w^0; the concentration factor (CF) depends only on the biological values of radionuclide metabolism, being independent both of Λ_w^0 and of λ. Maximum value reached by CF is $CF_{max} = \frac{I}{\beta}$.

Evaluation of "I" was made by a numerical method, by introducing into equation (1), written conveniently, the values of the known parameters of our experiments: $V_f/V_w = 2.5 \cdot 10^{-3}$, $T_b = 110$ days, $T_r = 65$ days. We have:

$$\frac{\Lambda_f(t) \cdot 10^{-3}}{\Lambda_w^0} \cdot e^{0.0102 \cdot t}\left(2.5 + 6.3 + \frac{1}{I}\right) = 1 - e^{0.0063\, t} \cdot e^{-0.00251 \cdot t}$$

For each pair of experimental values [t and $\Lambda_f(t)$] introduced into the equation, "I" was considered an unknown variable and the two members of the equation were established depending on it. The solution for "I" is the abscissa corresponding to the intersection of the two curves.

b) The case of a constant (chronic) contamination of water. The equation of migration (1) of radioisotope from water into fish is considered

valid here too, but there occur different conditions as regards C water activity that remains constant: $\Lambda_w(t) = \Lambda_w ct$ = constant, $\Lambda_f(0) = 0$.

Under these circumstances, the specific activity in fish is given by:

$$\Lambda_f(t) = \frac{I \cdot \Lambda_w ct}{\beta + \lambda} [1 - e^{-(\beta+\lambda)\cdot t}] \quad (2)$$

The maximum value of the specific activity in fish (Λ_f) tends to the plateau given by:

$$\Lambda_{f\,max} = \frac{I \cdot \Lambda_w ct}{\beta + \lambda}$$

The saturation of concentration factor: $CF = I/(\beta + \lambda)$ depends on "λ". "I" was simply obtained from equation (2) by introducing experimental values for "t" and $\Lambda_f(t)$.

EXPERIMENTAL RESULTS

The concentration of ^{85}Sr in fish *Pseudorasbora parva* for the two kinds of water is presented in figure 2. In both instances, the activity in fish increases rapidly up to the 20th day. For water unique contamination, the values of fish activities continue to increase, but not very much, and reach a maximum of 6,071 pCi/g on the 29th day. The concentration values for water chronic contamination increase and exceed those for the unique contamination, so that on the 50th day they reach 7,177 pCi/g.

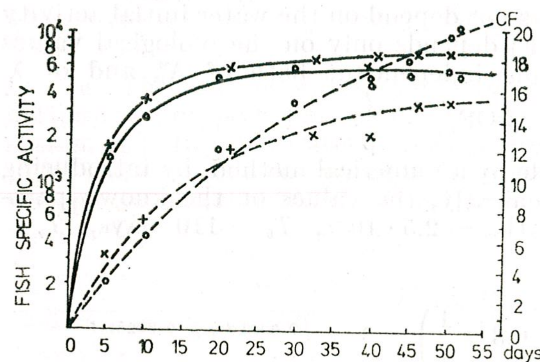

Fig. 2. — Dynamics of radiostrontium concentration in *Pseudorasbora parva* (——) and of concentration factor (— — —) in case of water unique (o) and chronic (×) contamination — experimental curves.

The concentration factors for both contaminations increase constantly during the experiment; the values for the unique contamination exceed those for the chronic one.

In the experiment of unique contamination with various activities (Fig. 3), fish reach, on the 20th day, activities proportional to water initial activity, within the limits of the experimental errors. Thus, fish

in water with 500 pCi/ml are 1.83 times more active than those in water with 250 pCi/ml, 4 and 8 times more than those in water with 150, and respectively, 50 pCi/ml. Therefore, ^{85}Sr concentration in fish, which originates directly from water through branchiae (absorption) and scales (adsorption), is proportional to water activity and it may be described by the equation: $\Lambda_f = 7.6 \cdot \Lambda_w^0$, where Λ_f = fish specific activity and Λ_w^0 = water initial specific activity.

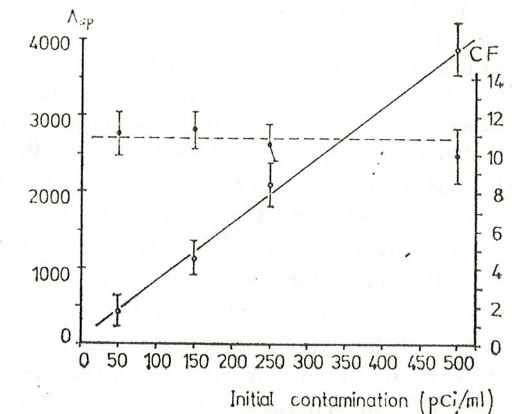

Fig. 3. — Variation of ^{85}Sr concentration by gold-fish *(Carassius auratus gibelio)* (o — o) and of concentration factor (● — ●) on the 20th day after water contamination depending on the initial activity of the aquarium solution.

DISCUSSION

Theoretical and experimental curves for unique and chronic contaminations (Figs 1 and 2) show a close likeness both as regards fish activity and concentration factors. For both contaminations, concentration in fish increases rapidly in the first days; for acute contamination a maximum is reached, followed by a slow decrease of fish activity that mainly indicates the physical disintegration of radionuclide besides a low concentration tending to saturation. In the case of chronic contamination, radioisotope concentration in fish tends to a plateau signifying the equilibrium between the isotope contribution in the environment and elimination by disintegration and metabolism.

Taking into consideration the fact that the half-time of ^{90}Sr-fission product, one of the most dangerous, is 157 times higher than that of ^{85}Sr, we can consider that a water unique contamination with ^{90}Sr is close to a water chronic contamination with ^{85}Sr. It allows the extrapolation of the results obtained in chronic contamination with ^{85}Sr to the case of unique contamination with ^{90}Sr, which has a great practical importance.

Confronting of experimental data with the equations deduced on the basis of the theoretical model allows the evaluation of water quantity filtered (I) from which fish take the radioisotope integrally. The direct dependence of Λ_f on Λ_w^0 (Fig. 3), found experimentally, suggests that the hypothesis of "I" independence of "Λ_w^0" (equations 1 and 2) is correct. The "I" values, calculated on *Carassius auratus gibelio* on the basis of experimental data, oscillate between 0.42 and 0.44 ml water/g fish per day.

The calculation of "I" on *Pseudorasbora parva* for different times of experiments shows, in both cases, a possible variation of "I" with time. The sizes of "I" show a group of higher values in the first part of the experiment, and another group of lower values, found in its last part. The decrease of "I" with time could be due to isotope adsorption, at the scales level, more accentuated at the beginning of the experiment. The fact that "I" represents a resultant of the radionuclide migration through more compartments with specific "I" (water-blood-bone, etc.) is equally important.

Though the initial, simplifying assumption of "I" constant in time cannot be rigorously justified, it offers the possibility of evaluating the limits between which "I" can vary. The differences occurring between "I" calculated from the experiment of unique contamination (Fig. 3) with more activities ($I = 0.43$ ml water/g fish per day) and the values of "I" given in table 1 may be due to the different fish species experimented, i.e. *Carassius auratus gibelio*, respectively *Pseudorasbora parva*, and to their different size, 25 — 30 g as compared to 5 — 7 g.

The calculation of "I" allows the evaluation of the maximum concentrations that can be reached as well as of the concentration factors. Thus, for the most probable values of "I", obtained by the end of the experiment, there were calculated values for more parameters, on the basis of the theoretical equations:

a) in the case of a unique contamination, for $I = 0.50$ ml water/g fish/day, we deduced the time in which the maximum radionuclide concentration in fish was obtained: $t_{max} = 71$ days, $\Lambda_{f\,max} = 6{,}400$ pCi/g to which C.F. = 29 corresponded, and the maximum C.F. at the plateau was about 79;

b) in the case of a chronic contamination, for $I = 0.45$, Λ_f reached the maximum value (at the plateau) of 13,200 pCi/g and C.F. = 26.5.

In figure 2 there can be seen that the above values obtained by calculation are close to those performed experimentally.

For the data introduced into the calculation program (the measurements performed on the 5th, 10th, 39th, 45th and 50th day) the dispersions of the results can be considered equal, and the averages of fish specific activities for both contaminations (unique and chronic), differ significantly with the probability of 99.9 per cent.

In conclusion, water chronic contamination induces in fish a radioisotope concentration significantly higher than water unique concentration, for the same initial activity.

The interpretation of the experimental data on the mathematical model suggested permits the evaluation of the water quantity filtered by fish (I) from which the radioisotope is integrally retained. The knowledge of this parameter allows the prognostication of time evolution of radionuclide concentration for other experimental conditions regarding water initial activity, radioisotopes and water volumes as well as the obtaining of t_{max}, $\Lambda_{f\,max}$, CF, etc.

Table 1

Variation of "I" (ml/g fish/day) depending on time in *Pseudorasbora parva*

Day	5th	10th	19th	22th	29th	34th	39th	45th	50th
Unique contamination	0.62	0.64	0.70	—	0.64	—	0.50	0.50	0.55
Chronic contamination	0.84	0.70	—	0.59	—	0.49	0.42	0.49	0.46

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C. LITEANU, S. GOCAN, *Cromatografia de lichide cu gradienți* (La Chromatographie des liquides à gradients), Editura Tehnică, Bucarest, 1976, 333 pages, 203 figures.

La chromatographie, comme méthode de séparation de différents composants existant dans un mélange complexe, prend une extension de plus en plus grande. A sa base réside l'existence d'une répétition de l'équilibre entre deux phases, qui peut se produire très rapidement et plusieurs fois, dans un intervalle de temps assez court.

En utilisant des gradients différents, on a la possibilité de répéter l'équilibre phasique, en passant de l'un à l'autre, dans des conditions expérimentales bien déterminées, ce qui a de grands avantages au point de vue de la précision des résultats. C'est pourquoi la méthode chromatographique des liquides à gradients a une utilisation de plus en plus large dans tous les laboratoires qui font des analyses chimiques, biologiques, pharmacologiques, médicales, alimentaires, hydrologiques, minières, etc.

Le présent volume s'occupe seulement de la chromatographie en phase liquide, en exposant — dans la première partie — la théorie générale du phénomène et surtout la technique du travail : le déplacement de l'éluent, du spot, de la réproductibilité des valeurs de Rf et les méthodes de calcul, pour pouvoir estimer l'efficience de la séparation et de l'optimisation du processus chromatographique.

Dans la deuxième partie on traite de l'utilisation des différents gradients : ceux de la phase mobile, de la phase stationnaire, du milieu et des gradients combinés.

Pour les laboratoires susmentionnés, le travail du professeur C. Liteanu et du dr. C. Gocan constitue un livre absolument nécessaire parce qu'il permet d'interpréter les résultats chromatographiques.

Le livre a un niveau scientifique très élevé qui nécessite des connaissances très approfondies de mathématiques. Je me permets de féliciter l'Edition Technique de Bucarest pour le soin avec lequel est présenté ce livre, paru en 1974 en Grande-Bretagne.

Eugen A. Pora

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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

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