REVUE ROUMAINE DE
BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

TOME 26 Nº 2

juillet — décembre 1981

SOMMAIRE

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, Nº 2, P. 109—188, BUCAREST, 1981

CONTRIBUTION TO THE KNOWLEDGE OF THE MONOKONOPHORA (CRUSTACEA, TANAIDACEA) OF THE EASTERN AUSTRALIAN CORAL REEFS

BY

MIHAI BĂCESCU

Etudiant un matériel de Tanaidacés des récifs coraliens de l'Ile Heron (au nord de Brisbane) envoyé par Dr. A. J. Bruce, l'auteur décrit trois espèces nouvelles : *Synapsuedes australianus*, *Pagurapseudes abrucei* et *Macrolabrus boeri* ; la première et la dernière espèce sont les premiers représentants des genres en question signalés des eaux d'Australie.

Material. Some representatives of the suborder Monokonophora from the material brought up on a fishing line from about 30 m deep, in the midchannel, from the Heron Island-Wistari Reef Channel by R. Boer and B. Hensley, on October 2, 1980. Thanks are due to Dr. A. J. Bruce, Director of Heron Island Research Station.

In this material, I found the following species :

1. SYNAPSEUDES AUSTRALIANUS n. sp.
(Fig. 1 A-I)

Diagnosis. Abdomen four-segmented, with sharp pleotelson ending in a bicuspide tip. Frontal part slightly excavated, with short crenellations. Antennula with strong digitations on the inner side of the first basal article and with a massive inner spine on the middle article. Chelipeds symmetrical in ♀. Antenna with slight inner denticulation on the proximal article and with a single long seta at the tip of flagellum.

Material. 1 ♀ with embryos, 1 ♀ with marsupial sheets and 1 juv. = 1.25, 1.0 and respectively 0.90 mm.

Description of adult female. Size : 1.25 mm. Body elongate, with soft integument, bears rare but strong hairs dorsally and especially on the sides of carapace and thoracomers (Fig. 1 A). Carapace with ± parallel and smooth sides (rare short hairs) ; rostrum a bit excavated and provided with slight denticulations (Fig. 1 B) ; ocular lobes pointed, eyes with lots of transparent omatidia buried in a central almost black nucleus.

Thoracomers ± equal in width, the last being a bit narrower (Fig. 1C) and longer, each with one or two strong central hairs. Pleon narrower, made of 3 short segments the first with 2 long lateral setae, without a belt of hairs ; pleotelson longer than the 3 segments, ending by a sharp tip with a crest bearing two spines (Fig. 1 D). The spine — better called

posterior tubercle — can hardly be seen in the small female ; it only shows 2 lateral hairs. A strong epistomal spine.

Antennula (Fig. 1 A), strong characteristic of the genus, is made of a 3-segmented basis — the basal segment with strong denticulations

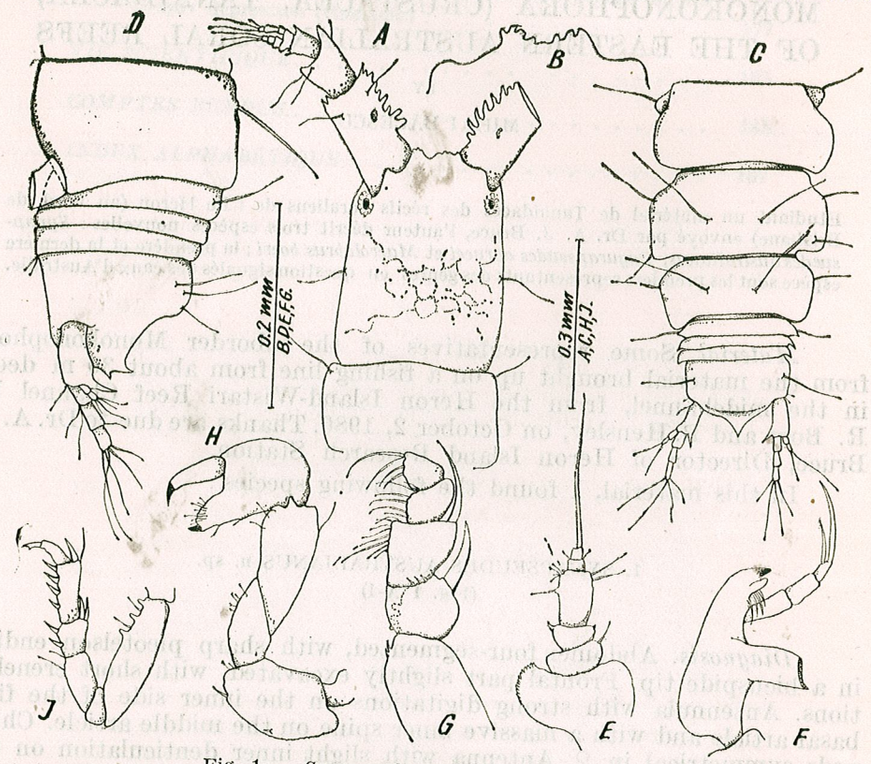


Fig. 1. — *Synapseudes australianus* n. sp. ♀
A, Carapace and A_1, seen tergally ; B, rostral area (front) ; C, posterior side (dorsally) ; D, ditto, lateral view ; E, antenna ; F, mandible ; G, maxilliped ; H, cheliped ; I, peraeopod II.

like digitations — while the middle segment ends by a big inner spine. Flagelli 3-segmented (without counting the basal segment common to both of them).

Antenna (Fig. 1 E) with basal article slightly denticulated innerly (rather hirsute), with 3-segmented flagellum ending by a strong simple seta almost as long as A_1.

Mandible (Fig. 1 F) with a narrow *pars molaris* and curved basis. Maxilliped (Fig. 1 G) with 3 retinacles and strong outer spines on segments III and IV ; chelipeds equal (Fig. 1 H) with black tips of chela. Peraeopods II and III with 4 spines on the tergal edge of propodus (juv. ♀ shows only 2 spines) and 3 tubercles on the back of basis (Fig. 1 I). Uropods with a strong spiny dorsal prolongation of the basis, with 3-segmented endopodite and 2-minutely-segmented exopodite (Fig. 1 D).

Colour : transparent white with strong brown areas on the middle of carapace, on the fourth thoracomer and a few brown spots on the sides of thoracomers ; the middle segments of the basis A_1, the distal part of carpus of peraeopod II and the basis of mandible of violaceous brown colour.

Holotype ♀ (transected) under no. 522 in the collection of the "Grigore Antipa" Museum. Paratype 1 juv. ♀, ibid., under no. 523. The collecting area is that mentioned at the beginning.

Observations. Synapseudes australianus is the first representative of the genus found in the Australian waters and the second one cited for the western Pacific Ocean, its occurrence in the coral reefs of Heron Island and the violaceous spots scattered on its body, confirm the adaptation to the corallicolous life of these Tanaids in all areas with coral reefs. I mention that Lang (1970) figures a *Synapseudes* sp. (Pl. I, Fig. A) of New South Wales, that seems to belong to the same group as our species, but only specifies that it has 4 abdominal segments, giving no other details.

Among the 13 species belonging undoubtedly to this genus, the new species—the 14th— approaches the most *S. violaceus* Băcescu 1976 b of the Tanzanian corals, showing also 4 pleonites and the same distribution of the coloured spots, reminding also of *S. setoensis* Shiino 1951, of the Japanese superlittoral, by the armature of maxilliped and of peraeopod II.

As I had not the male, I do not know if it also shows dimorphism or asymmetry of chelipeds. Anyhow, *S. violaceus* is distinguishable by such a rostrum and by the apical crest of pleotelson that has 5 teeth, while *S. setoensis* can be first distinguished by the 3-segmented pleon by the lack of tubercles on basis A_1 and by the presence of 2 short terminal setae on A_2.

2. PAGURAPSEUDES ABRUCEI n. sp.
(Fig. 2, A-L)

Diagnosis. Tanaid with triangular rostrum without denticulations, with two pairs of biramous pleopods (♂ ♀), 5—7 spines on the inner side of basal article of A_1 ; segm. III of maxilliped with 2-spines on each side ; chelipeds symmetrical in ♀ and asymmetrical in ♂ (the right one larger) ; uropod dimorphic with 3-segmented endopod in ♀, 2-segmented in ♂.

Material. 1 ♂ and 2 ♀♀ with marsupial sheets.

Description of the female. Integument soft, ivory, with rare and long simple setae on all segments. Carapace only a bit longer than wider, with triangular (?) rostrum (Fig. 2 A). Hyposphenia are missing, excepting one very small between peraeopods II.

Sides of thoracomers without spines or denticulations. Abdomen with lateral hairy setae. Ocular lobes triangular ; eye like a crown of omatidia thrust into a black pigmentary mass.

Antennulae (Fig. 2 A) with 6—7 fine spines on the inner side of the first basal segment ; outer flagellum 4-segmented, the inner one 2-segmented also counting their small apical article (Fig. 2 A), arrow, not the proximal common one).

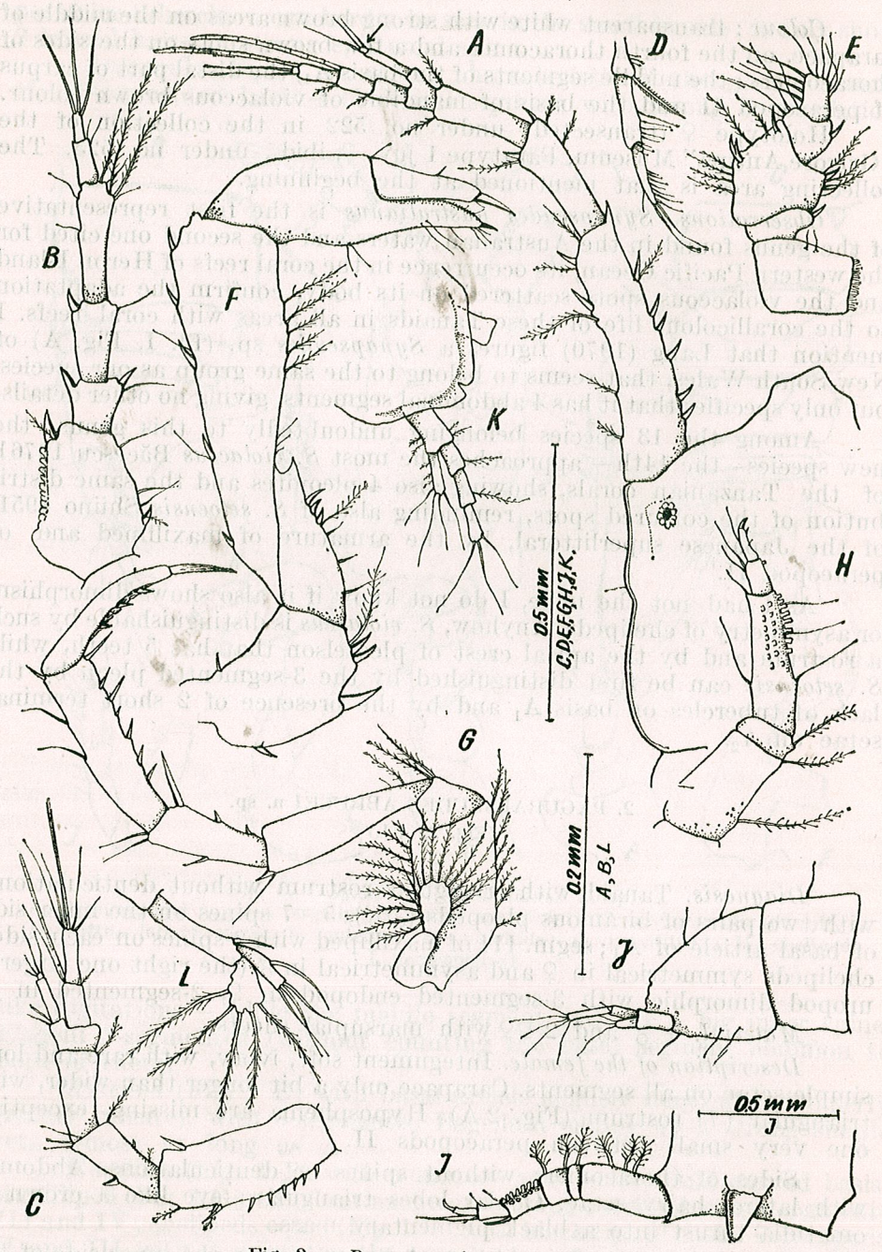


Fig. 2. — *Pagurapseudes abrucei* n.sp.
A, B, E, F, H, K = ♀; C, G, L, I, J = ♂
A, Carapace and A_1, tergal view ; B, antenna ; C, antennula ; D, mandibular palp ; E, maxilliped ; F, cheliped ♀; G, peraeopod II ; H, peraeopod V ; I, last peraeopod and genital cone ; J, extremity of pleon and uropods, lateral view ; K, id. ♀ tergally ; L, pleopod I.

Antenna with first basal article wide, vaguely denticulated on the inner side terminating by a spine of the same size as the outer one (Fig. 2B).

Mandibular palp-like in Fig. 2 D.

Maxillule common to the genus. Maxilliped with segm. III much larger than the other segments, with 2 spines on each free side.

Chelipeds symmetrical, with the tip of fingers brown ; carpus with 6 tergal spines ; ischium with a characteristic distal excavation ending by a spine ; basis with 4 sternal spines. Peraeopod II with an immense biarticulated ± rectangular exopodite (Fig. 2 G), with setae twice as long as the width of basis. The rest of peraeopods with the morphology characteristic of the genus (Fig. 2 H) with 4 series of suckers.

Two pairs of biramous pleopods with oval exopodite a bit shorter than endopodite ; they are perfectly similar, but the basis of pleopod I is longer (Fig. 2 L).

Uropods. attached subterminally, with 3-segmented endopodite and 1-segmented exopodite (Fig. 2 K). The studied female was shedding its coat and measured 3.2 mm.

Description of the male. The only available male had the frontal part broken. It differed from the ♀ in the size dimorphism of chelipeds, in the smaller size (only 2.2 mm), in the 2 segmented big flagellum of A_1 (Fig. 2 C) with 2 aesthetascs, in the armature of plumose setae on the tergal edge of the plate of basis (that is nearly two times shorter than in ♀) and, of course, in the long genital cone on the middle of the last sternite (Fig. 2 I).

It shows a tiny hyposphenial spine only on the sternite of the first free thoracomer. Extremity of abdomen like in Fig. 2 J, with a 2-jointed endopodite.

Holotype : a transected ♀ — under no. 524 — in the collection of the "Grigore Antipa" Museum ; allotype ♂, ibid., no. 525.

Observations. Although the first representative of the genus was described in 1901 by Whitelegge from the Australian waters, no other species was cited from there. But the generotype *P. spinipes* distinctly differs from the new Australian species in the bicuspide denticles of the proximal segment of the basis of antennula, in the shortness of the abdominal segments and especially in the cut off frontal part and the varied number of pleopods (0—3).

P. abrucei is a conchiliolous species, preferring the shells of Cerithidae ; it is characterized within the group of 8 species known so far as belonging to the genus by the presence of 2 pairs of biramous pleopods (with long peduncles) corresponding to the first and second pleonites, as well as by the special shape of rostrum, of maxilliped and the armature of chelipeds.

3. MACROLABRUM BOERI n. sp.
(Figs. 3 and 4)

Diagnosis. Pagurapseudid with a long spiniform epistome much exceeding the frontal part ; 2 pairs of biramous pleopods (pairs I and II), with strong exopodite of peraeopod II and a strong armature of denticules

not only on the sides of carapace, but also on antennae, mandible and maxilliped. Uropods with thick finely serrated setae curved at their tip for better seizing.

Material. 1 adult ♂ and 1 adult ♀ from loc. cit.

Description of the female. (Fig. 3). Its shape resembles that of the *Pagurapseudes*, with integument soft and sticky but lightly calcified and slightly waved on the carapace; the latter shows a strong lateral denticulation alternating with short plumose setae and a triangular frontal part bordered by two strong lateral prominences (approximately above basis A_1), ending by three anterior teeth and much exceeded from below by the strong epistomal spine (Fig. 3 B and arrow e, Fig. 3 C). Eyes well defined. Thoracomers ± smooth; on the back of each thoracomer a big seta flanked by 2 small ones.

Antennulae with spiny basis and big 4-segmented flagellum with 4 aesthetascs, the small 2-segmented, both of them being fixed on the same

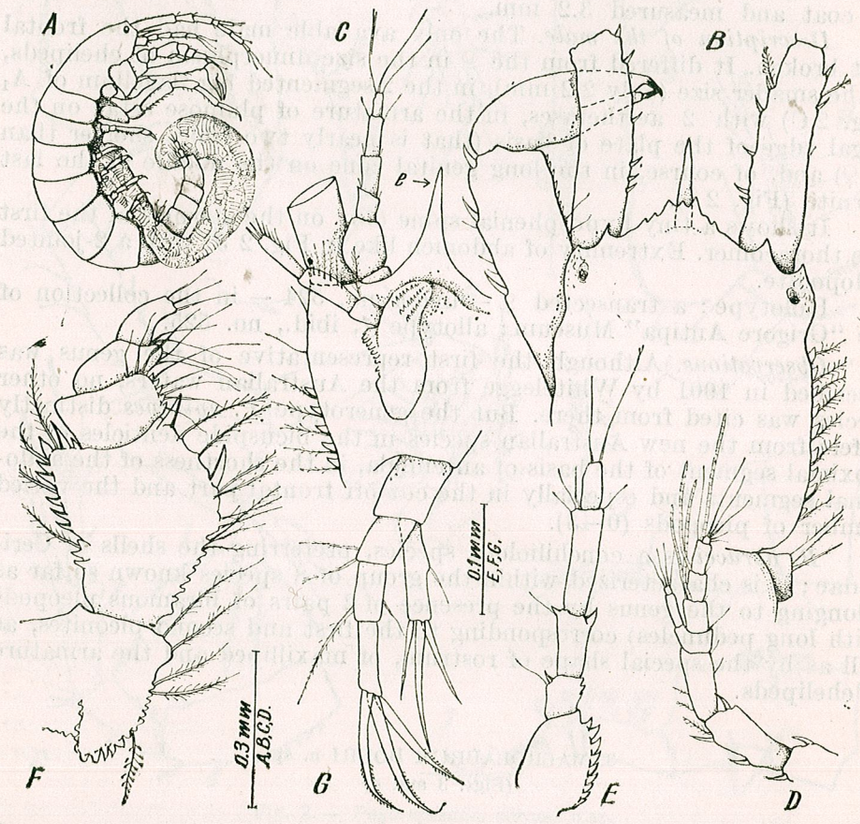


Fig. 3. — *Macrolabrum boeri* n.sp. ♀
A, ♀ with sack-shaped marsupium; B, anterior side, tergal view; C, anterior side, ventral view; e, enormous spine of labrum (epistome); D, antennula; E, antenna; F, maxilliped II; G, uropod.

basal article (Fig. 3 D); when we say 4- and respectively 2-segmented, we also take into consideration their small apical segment, without the big common basal one.

Antenna with proximal oval discoidal article with strong outer denticulation (Fig. 3 E).

Mandible also with strong teeth on the basis and on pars masticatoria (Fig. 3 C), with 3-segmented palp. Maxillae of ordinary type; teeth of maxilla II are brown. Maxilliped (Fig. 3 F) shows denticulations on segments III and IV (especially on the sternal edge) that become real spines here and there.

Chelipeds symmetrical, with large spines on carpus (Fig. 3 B) on tergal face and small spines on the sternal face. Tip of fingers and their prehensile edge of brown colour.

Peraeopod II and the rest of peraeopods like those from ♂; on peraeopods III and IV long oval marsupial sheets.

Uropod is laterally and inferiorly attached to pleotelson and is formed of a uniramous exopodite and a 3-segmented endopodite, both ending by 3 strong and shortly serrated setae, curved at their tip (Fig. 3G). *Very characteristic of the female is the enormous bag in which the marsupium prolongs*; this cylindrical bag follows the spiral of the abdomen exceeding it (Fig. 3 A) in length and diameter. I found in it 13 embryos with sketched appendages but without eyes.

Description of the male. (Fig. 4) Of same size as ♀ (3.2 mm) it shows the same morphology of the segments of the body; only the frontal part is different, having on its tip a bunch of 3 horns (Fig. 4 A and B, arrow); epignath stronger.

Labial lobes hairy, with 2 distal little spines (Fig. 4 C).

Antennae, mouth parts and pleopods like in ♀. Setae simple but strongly agglutinant, as long as 1/2 of the diameter of the respective segment, one on each thoracomer and 2 flanked by simpler setae on pleonites.

Chelipeds asymmetrical (Fig. 4 E and F) the right one being slightly stronger, particularly its chela; carpus with a double armature of denticules on sternal face; the outer face extending apically like a crown of spines near the articulation of propodus (Fig. 4 F); on tergal side 3 + 2 spines on big paw and only 2 distal ones on the small right paw (Fig. 4 E). Meros with distal spine apparently characteristic of Australian Pagurapseudidae (Fig. 4 F, arrow) and 3 + 1 strong spines on the basis.

Peraeopod II (Fig. 4 G) prehensile shows the morphology typical of these conchilicolous Tanaids, i.e. a widened basis with a strong armature of hairy setae longer than in ♀ and a strong 2-segmented exopodite like a fan with at least 15 plumose rays, an ideal organ of airing the body screwed up in the shell. The remainder of peraeopods show 2 — 3 rows of tubercles-suckers: on propodus-carpus and meros of anterior peraeopods (Fig. 4 H) and practically only along the carpus of the posterior peraeopods (Fig. 4 I).

Pleopods I and II (Fig. 4 D and J) subequal, wide hairy blades that contribute to airing the space inside of the shells.

Hyposphenia weak, with the exception of the big genital cone from sternite VII (p, Fig. 4 D).

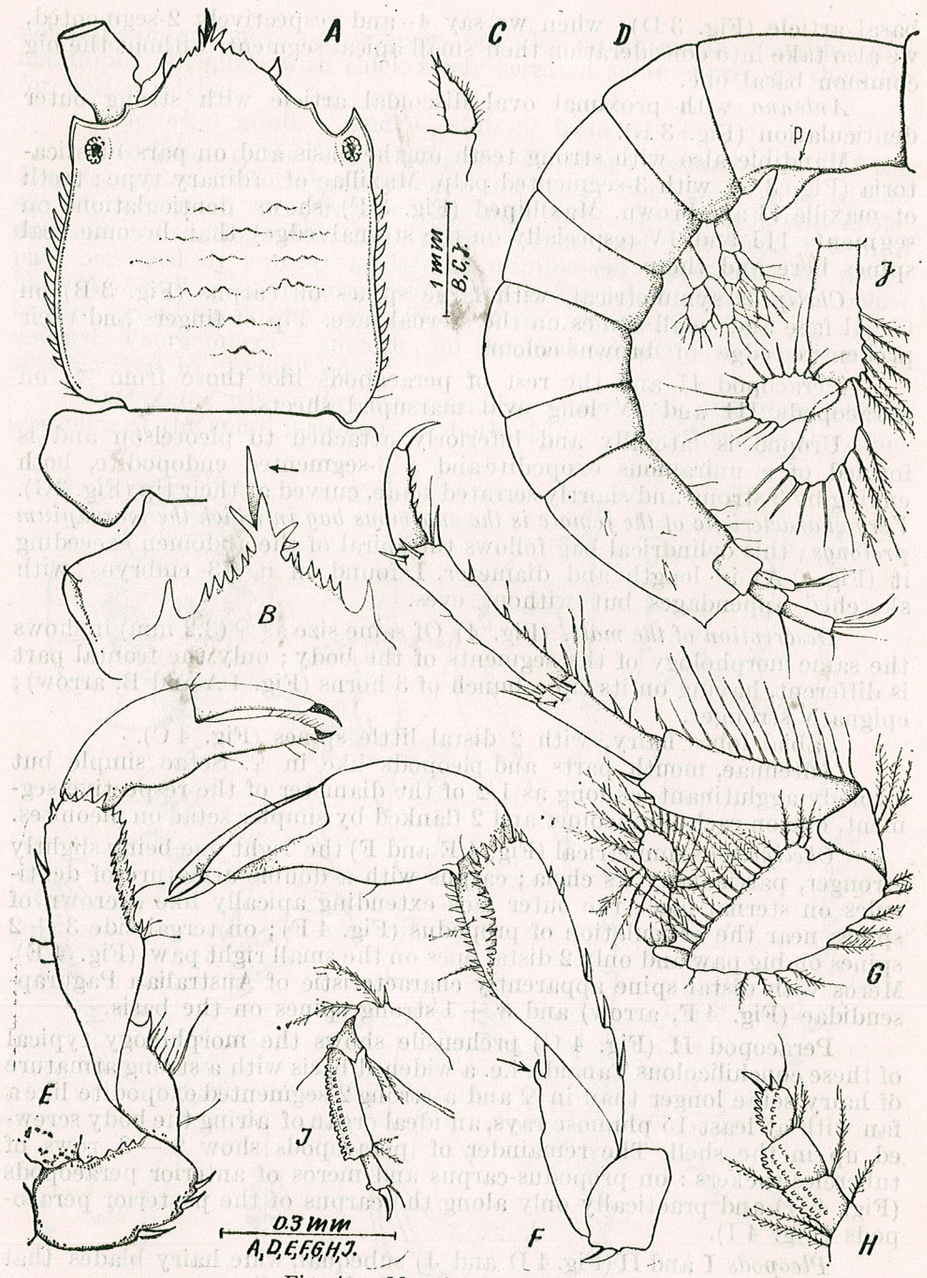


Fig. 4. — *Macrolabrum boeri* n.sp. ♂
A, anterior side, tergal view; B, its rostrum, magnified, arrow = epistomal spine; C, a lobe of labium; D, abdomen, lateral view; p, penial cone; E, right cheliped; F, left cheliped; G, peraeopod II; H, peraeopod III, I, peraeopod VII; J, pleopod II.

Uropods with 2-segmented endopodite (as I had a single ♂, I do not know if this reduction of segments is a dimorphic feature). The holotype ♂ of *M. boeri* is deposited at the "Gr. Antipa" Museum under no. 526; allotype (transected ♀), under no. 527.

Observations. The bag-shaped spiral marsupium contained 13 embryos with sketched appendages, but without eyes and with a parasite copepod.

It is the first representative of genus *Macrolabrum* Băc. (1976 a) that is mentioned in the Australian waters; it differs from the only species of the genus known so far (in the Tanzanian waters) in the non-serrate uropodal setae, the non-reduction of the articles of flagelli of antennae and in the finer and smoother epistome.

Seen in its shell, with chelae stooped over the aperture and with peraeopods II distant, the strong spine of labrum, that is clearly seen from under the rostrum, appears like a typical defensive organ.

As regards the number of segments of big flagellum of antenna in these conchilicolous Tanaids that seems to differ by 1—3 units with some species (according to some authors), one should specify the way of counting: *with* or *without* the basal article (segment) common to both flagelli, which is considered by certain authors as the first article of the big flagellum. Likewise, the tiny apical article of both (clearly distinguished only with great magnifiers) near which is based the distal aesthetasc, seems to be ignored when counting the articles of flagelli of antennulae.

Without being too rich in phanerae, the integument of both representatives of family Pagurapseudidae shows a sticky characteristic secretion particularly on the appendages that agglutinates on the spot any silt particle.

It seems that the bag shape of marsupium, so clearly shown in our species, is also common to other species of *Pagurapseudes* (at least, to some of them), judging by the only existing sketch — that of Bouvier — for *P. bouryi* (see Fig. 1, p. 13 Bouvier); anyhow, it is a biomorphological adaptation to the life in the whirls of the shells, maybe even more important than the twisting of abdomen or the adaptation of peraeopods III—VII to hanging on and of peraeopod II to airing. The whole ethology of the Pagurapseudidae deserves a careful study.

The presence of two pairs of biramous pleopods both in *P. abrucei* and *M. boeri* indicates populations of a more primitive type in the Australian corals than in the Caribbean ones, with pleopods reduced as far as number and development are concerned: only a pair of non-biramous pleopods (*P. guitarti* Guţu) or reduced to a mount (*P. bouryi*) or even disappeared (*P. laevis* Menzies). Intermediary would be the species of the western Indian Ocean with a single but biramous pair of pleopods (*P. varians*); it is true that in this species too, pleopods (II) are reduced, which indicated the evolution line in the reduction of these appendages.

Synapseudes australianus is the first representative of the genus signalled out in the Australian waters, exactly like *Microlabrum boeri*. Taking also into account *Pagurapseudes spinosus* and *P. abrucei*, the number of these coralicolous Tanaids increased to four in the Eastern Australia.

REFERENCES

1. Băcescu M., 1976, a, Rev. Roum. Biol.—Biol. Anim., **21**, *1*, 3—11.
2. Băcescu M., 1976, b, Trav. Mus. Hist. nat. "Gr. Antipa", **17**, 51—63.
3. Bouvier E. L., 1918, Bull. Mus. Hist. Nat., **24**, 12—15.
4. Brown A. C., 1956, Ann. Mag. Natural Hist., Ser. 12, **11**, 518—586.
5. Guţu M., 1976, Trav. Mus. Hist. nat. "Gr. Antipa", **17**, 85—91.
6. Lang K., 1970, Ark. zool. (*2*), **22**, 595—626.
7. Shino S. M., 1951, Rep. Fac. Fisheries, Prefectural Univ. of Mie, **1**, 12—25.
8. Whitelegge Th., 1901, Austral. Mus. Mem., **26**, *2*, 203—225.

Received November 28, 1980

Museum of Natural History "Gr. Antipa"
Bucureşti, Kiseleff 1

NOUVELLES ESPÈCES D'*ORIBATES* *(ACARINA, ORIBATIDA)*

PAR

MAGDA CĂLUGĂR et N. VASILIU

In this paper the authors describe two species new for science belonging to the ord. *Oribatida* (Dugés, 1835):
1. *Phthiracarus flexipilus* n.sp. is characterized by: elongate sensillus, curved, like a sickle; the adanal hairs are shorter than the anal ones.
2. *Ceratozetes ovidianus* n.sp. is characterized by: truncated rostrum; translamella is absent; the interlamellar hairs are short and thin; the dorso-sejugal suture is very acuminate.

Dans la présente étude nous décrivons pour la science, deux nouvelles espèces d'*Oribates* identifiées dans une collection provenue de l'Institut de sciences biologiques de Bucarest.*

Superfamille ***PHTHIRACAROIDEA*** Grandjean, 1954

Famille PHTHIRACARIDAE Perty, 1841

Phthiracarus flexipilus n. sp.

Diagnose. Le sensillus allongé et légèrement courbé a une forme de faucille. Les poils notogastraux très longs, fins et lisses sont flexibles. Les poils adanaux (ad_1; ad_2; ad_3) sont plus courts que les anaux (an_1; an_2).

Dimensions. Prodorsum: L — 137 μ; Notogaster: L — 266 μ; H — 192 μ. Volet génital: L — 92 μ; 1 — 42 μ. Volet anal: L — 100 μ; 1—37 μ. Poils du prodorsum: ss — 62 μ; la — 28 μ; il — 17 μ; ro — 30 μ; ex — 22 μ. Poils du notogaster: 55—56 μ.

Morphologie. Le rostrum (ro) arrondi. Les poils rostraux fins, lisses sont peu courbés et érectes. Dans la région interbothridiale, il y a les poils interlamellaires (il) qui sont fins, lisses et couchés sur la surface du prodorsum. Les poils lamellaires (la) situés en avant de la bothridie sont plus courts que les interlamellaires. Le sensillus (ss) long pédicellé et peu courbé est semblable à une faucille. Au voisinage de la bothridie s'articule une paire de poils exobothridiaux (ex.) qui sont effilés.

Le notogaster a 14 paires de poils flagelliformes (fig. 1).

La région ano-génitale (fig. 2) présente sur les volets génitaux sept paires de poils comme des petites épines. Les volets anaux se prolongent dans leur partie postérieure au-dessous du notogaster. Les deux paires

* Nous remercions notre collègue Viorica Honciuc pour ce matériel.

de poils anaux (an_1 ; an_2) sont plus longs que les trois paires de poils adanaux (ad_1 ; ad_2 ; ad_3).

Le cérotégument est pourvu d'une ornementation pointillée.

On trouve chez les auteurs un holotype et sept paratypes.

Terra-typica : fôret de hêtre Posada sur le massif Gîrbova, alt. 800 m (monts Bucegi). Biotope : litière. Date : le 10 septembre 1977 ; le 10 octobre 1977.

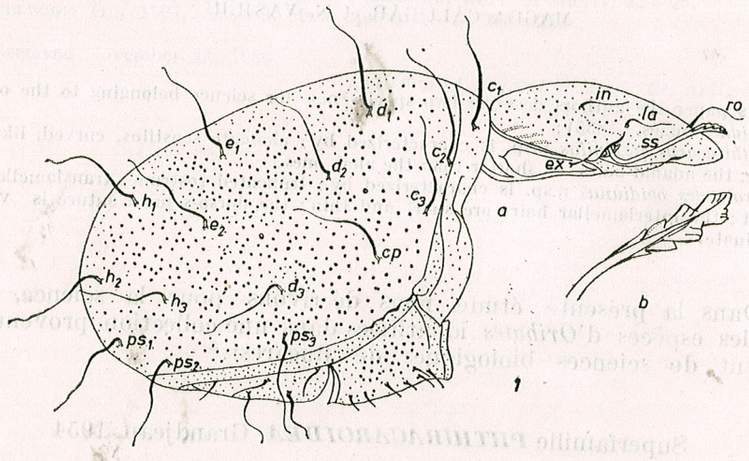


Fig. 1. — *Phthiracarus flexipilus* n.sp. : a, corps, vue latérale ; b, extrémité du sensillus.

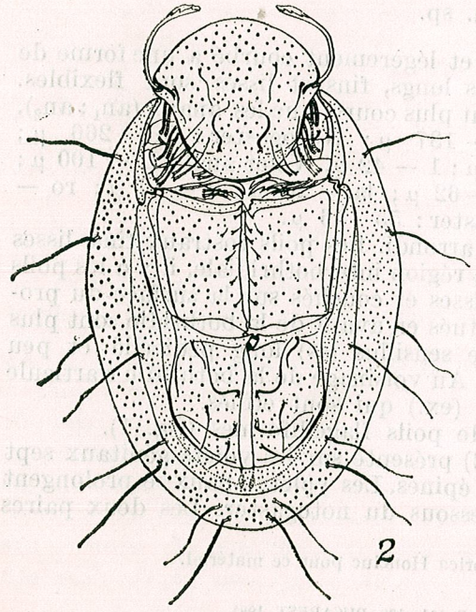
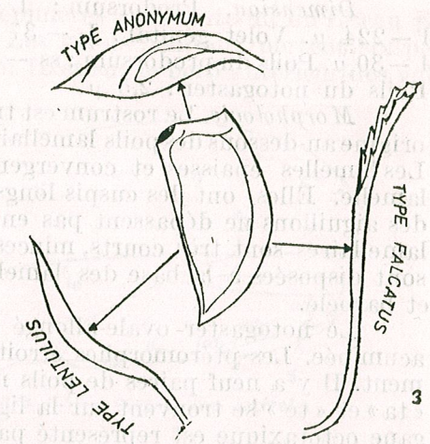


Fig. 2. — *Phthiracarus flexipilus*, corps, vue ventrale.

Fig. 3. — Les types du sensillus des espèces du genre *Phthiracarus* Perty, 1841 de Roumanie.



Remarques. Par la forme de faucille du sensillus *Phthiracarus flexipilus* n.sp. s'encadre dans le même groupe d'espèces que *Phthiracarus falcatus* Hammer, 1977 trouvé en Pakistan. Nous en concluons, que les 17 espèces du genre *Phthiracarus* Perty, 1841 identifiées jusqu'à présent en Roumanie appartiennent d'après le type du sensillus à trois groupes :

1. **Groupe anonymum** — le sensillus fusiforme ou fusiforme-arrondi, est représenté par les espèces suivantes : *Phthiracarus anonymus* Grandjean, 1933 ; *Phthiracarus piger* (Scopoli, 1763) ; *Phthiracarus globosus* C. L. Koch, 1841 ; *Phthiracarus nitens* Nicolet, 1855 ; *Phthiracarus crenophilus* Willmann, 1951 ; *Phthiracarus tardus* Forsslund, 1956 ; *Phthiracarus lanatus* Feider et Suciu, 1957 ; *Phthiracarus dubinini* Feider et Suciu, 1958 ; *Phthiracarus pallidus* Feider et Suciu, 1958 ; *Phthiracarus jacoti* Feider et Suciu, 1958 ; *Phthiracarus danubianus* Feider et Călugăr, 1970.

2. **Groupe lentulus** — le sensillus fusiforme — allongé ou fusiforme—filiforme, est représenté par les espèces suivantes : *Phthiracarus lentulus* (C. L. Koch, 1841), *Phthiracarus italicus* (Oudemans, 1906), *Phthiracarus ligneus* Willmann, 1931 (= Ph. sellnicki Feider et Suciu, 1957), *Phthiracarus parabotrichus* Feider et Suciu, 1957 ; *Phthiracarus baloghi* Feider et Suciu, 1957.

3. **Groupe falcatus** — le sensillus allongé avec la partie distale en forme de faucille — est représenté par *Phthiracarus flexipilus* n. sp. (fig. 3).

Superfamille CERATOZETOIDEA Balogh, 1961

Famille CERATOZETIDAE Jacot, 1952

Ceratozetes ovidianus n.sp.

Diagnose. Le rostrum est tronqué. La translamelle manque. Les cuspis sont longs et pointus. Les interlamellaires sont les plus courts et le plus minces poils du prodorsum. Le notogaster présente la suture dorso-séjugale très acuminée.

Dimension. Prodorsum : L — 111 μ. Notogaster : L — 310 μ ; l — 224 μ. Volet génital : L — 37 μ ; l — 24 μ. Volet anal : L — 75 μ ; l — 30 μ. Poils du prodorsum : ss — 42 μ ; la — 54 μ ; il — 13 μ ; ro — 55 μ. Poils du notogaster : 2,5 μ.

Morphologie. Le rostrum est tronqué. Les poils rostraux, qui ont leur origine au-dessous des poils lamellaires, sont fins, barbelés et convergents. Les lamelles épaisses et convergentes ne sont pas réunies par la translamelle. Elles ont des cuspis longs et pointus. Les poils lamellaires comme des aiguillons ne dépassent pas en longueur le rostrum. Les poils interlamellaires sont très courts, minces et lisses. Les bothridies peu évidentes sont disposées à la base des lamelles. Le sensillus recliné est fusiforme et barbelé.

Le notogaster ovale-allongé présente la suture dorso-séjugale très acuminée. Les ptéromorphes étroites et allongées sont disposées obliquement. Il y a neuf paires de poils notogastraux à peine visibles. Les poils « ta » et « te » se trouvent sur la ligne d'insertion des ptéromorphes. L'organe octotaxique est représenté par une seule paire d'aires poreuses disposée avant les poils « ta » (fig. 4,5).

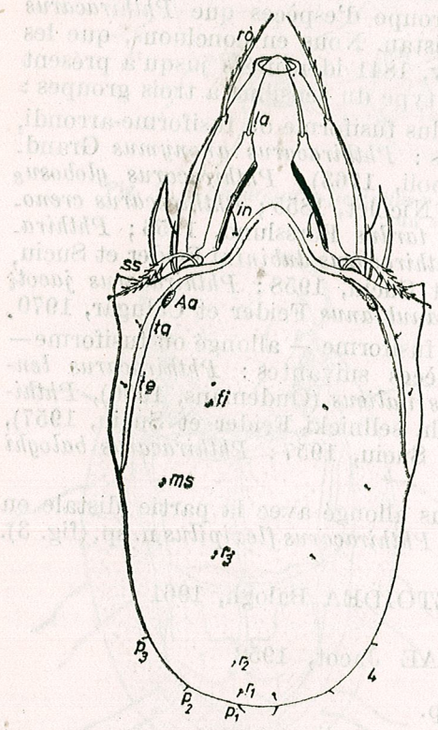


Fig. 4. — *Ceratozetes ovidianus* n.sp., corps, vue dorsale.

Sur la face ventrale, on observe les crêtes circumpédales étroites qui couvrent partiellement les tectopédies. Les apodèmes sont obliques. Les poils épimériaux, réduits en dimension, sont disposés selon la formule : 2—2—1—2. La surface des épimères 3 présente un fin réseau polygonal autour des volets génitaux. Les volets génitaux portent six paires de poils fins et effilés dont trois sont fixés sur le bord antérieur des volets,

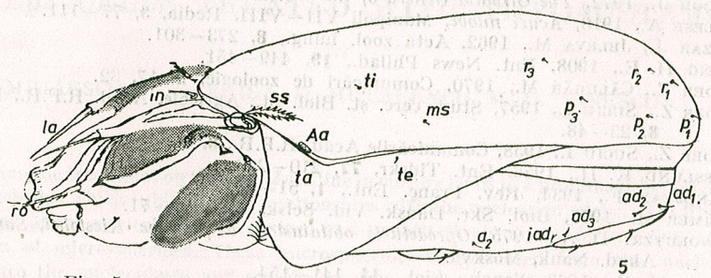


Fig. 5. — *Ceratozetes ovidianus* n.sp., corps, vue latérale.

une paire dans l'angle antéro-latéral et deux paires sur le bord postérieur. Derrière les volets génitaux, il y a une paire de poils agénitaux. Les volets anaux présentent deux paires de poils petits et lisses. Il y a trois paires de poils adanaux et une paire de lyrifissures (iad) disposées avant la première paire de poils adanaux (fig. 6).

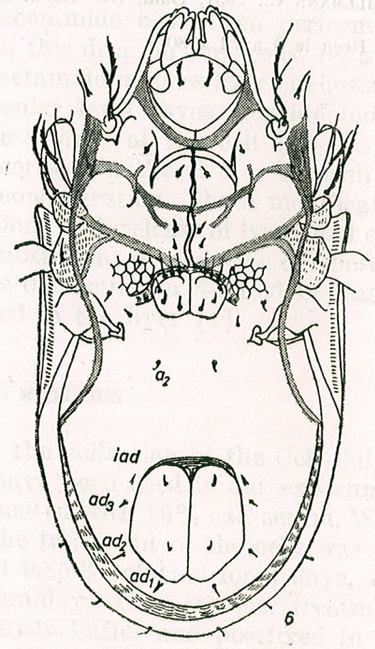


Fig. 6. — *Ceratozetes ovidianus* n.sp., corps, vue ventrale.

On trouve chez les auteurs un holotype et un paratype.

Terra-typica : La prairie xérophile près de Năvodari (dép. de Constanţa). Date : le 18 juillet 1979.

BIBLIOGRAPHIE

1. Balogh J., 1943, *Magyarorszag pancelosatkai (Conspectus Oribateorum Hungariae).* Mat. Termeszettund Kozlem, **39**.
2. Balogh J., 1972, *The Oribatid Genera of the World.* Akad. Kiádo, Budapest.
3. Berlese A., 1910, *Acari nuove,* Manipoli VII—VIII, Redia, **9**, 77—111.
4. Csiszar J., Jeleva M., 1962, Acta zool. hung., **8**, 273—301.
5. Ewing H. E., 1908, Ent. News Philad., **19**, 449—451.
6. Feider Z., Călugăr M., 1970, Comunicări de zoologie, **2**, 17, 32.
7. Feider Z., Suciu I., 1957, Stud. cerc. șt. Biol. St., Agricole, Acad. R.P.R., Filiala Iași, **8**, 23—48.
8. Feider Z., Suciu I., 1958, Comunicările Acad. R.P.R., **8**, *4*.
9. Forsslund K. H., 1956, Ent. Tidskr, **77**, 210—218.
10. Grandjean F., 1934, Rev. Franç. Ent., **1**, 51—58.
11. Hammer M., 1977, Biol. Skr. Dansk. Vid. Selsk, **21**, *4*, 1—71.
12. Krivolutzki D. A., 1975, *Opredeliteli obitaiustcih v potchive Klestchei, Sarcoptiformes.* Akad. Nauk, Moskva.
13. Menke H. G., 1963, Senck : biol., **44**, 141—154.
14. Menke H. G., 1964, Senck biol., **45**, 621—634.
15. Menke H. G., 1964, Senck biol., **45**, 635—640.
16. Mihelčič F., 1956, Zool. Anz., **156**, 5—6, 205—226.
17. Pérez-Inigo C., 1968, « GRAELLSIA » Rev. Entomol., Ibericos, **24**, 143—238.
18. Pérez-Inigo C., 1972, Biol. R. Soc. Española Hist. Nat. (Biol.), **70**, 185—206.
19. Pérez-Inigo C., 1972, « Eos » Rev. Española Entom., **47**, 247—333.
20. Pérez-Inigo C., 1974, « Eos » Rev. Española Entom., **48**, 367—475.
21. Schweizer S., 1956, Ergeb. Unters. Schweiz. Nat. Parks., N.F., **5**, 213—377.
22. Shaldîbina E. S., Uc. zap. Gorik., GPI, **114**, 25—50.
23. Willmann C., 1931, Dahl, Die Tierw. Deutsch., **22**, 79—200.

Reçu le 3 avril 1980

Université « Al. I. Cuza »
Iași, Calea 23 August 11

EXPERIMENTAL RIBOSOMAL GENE AMPLIFICATION IN HEp2 CELLS TREATED WITH THYOACETAMIDE

BY

GR. MIHĂESCU, L. GAVRILĂ, D. MIȘCALENCU, M. D. IONESCU

Thyoacetamide treatment on the HEp2 cellline in culture leads to a prominent hypertrophy of the nucleolus which exhibits close relationships with the nuclear envelope. The fibrillar component of the nucleolus is reorganized and quantitatively diminished leading to the formation of microspherules. These microspherules are released from the nucleolus, passed into the nucleoplasm and sometimes even in the cytoplasm. These events are considered in this paper in terms of ribosomal gene amplification phenomenon. The formation of fibrillar microspherules is correlated with an increase in the number of nuclear bodies. It was inferred that nuclear bodies might originate from fibrillar microspherules.

INTRODUCTION

Previous experiments using thyoacetamide have been performed in order to search for the *in vivo* effects of this drug on liver cells. By a prolonged administration in rat [5] thyoacetamide induces liver cirrhosis and cancer of biliary ducts. At the molecular level thyoacetamide induces disturbance in the ribonucleoproteinic system of the cell [6] and this effect is expressed by an abnormal increase in the size of nucleoli and a decrease of the cytoplasmic RNA concentration. These modifications are due to lysosomal membrane alterations with release of lysosomal enzymatic content in the cytoplasm and a subsequent degradation of ribosomal RNA and proteins [1]. Thyoacetamide determines a 5-6-fold increase of the level of albumin that is synthesized in the liver [4].

MATERIAL AND METHODS

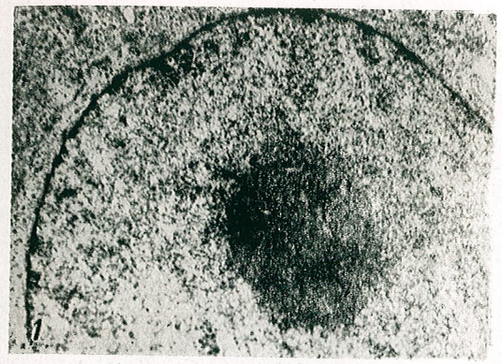
The cells of the HEp2 line from the collection of the Cell Culture Laboratory of the Virology Institute have been used in our experiment, being grown on Eagle medium supplemented with 10% calf serum. When the monolayer was almost complete, the treatment of the cells was performed using thyoacetamide in a NaCl 0.85% solution for 3 days, with a quantity equal with 150 mg/l medium/day. After the last treatment, the cells were fixed in 2% glutaraldehyde buffer and postfixed in 1% osmium tetraoxide. The thin sections have been stained with uranyl acetate and lead citrate and examined in a Phillips 201 EM.

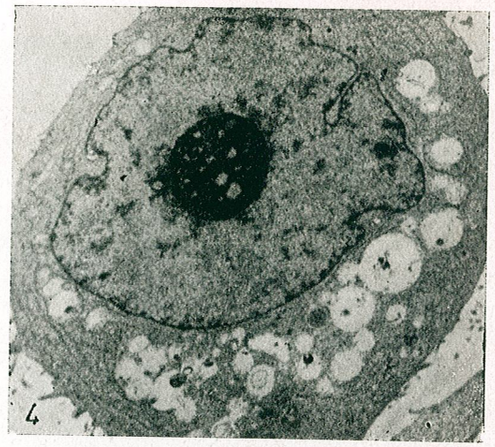
REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, Nº 2, P. 127—130, BUCAREST, 1981

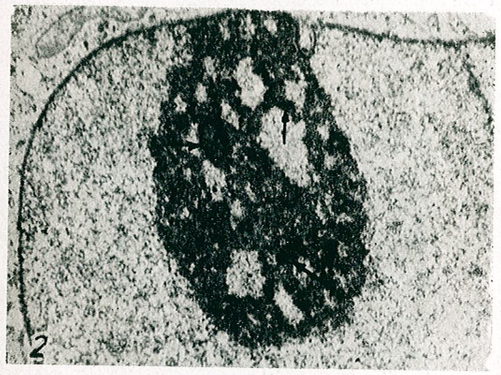
RESULTS

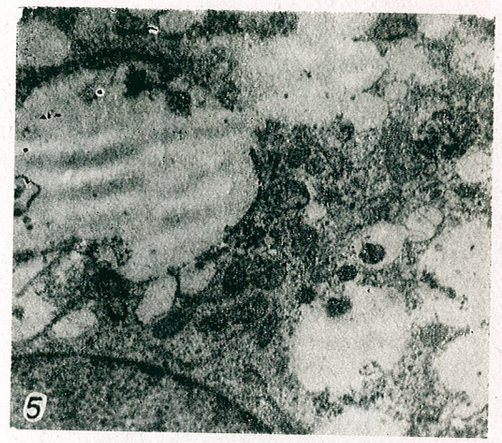
The untreated HEp2 line cells have an abundant cytoplasmic content with a high concentration of ribosomes — a peculiar feature of malignant cells with a high rate of growth. The nucleus has a round or oval shape; sometimes it exhibits deep indentations. The nucleolus, most frequently existing as a single body, has a usual size occupying a small portion of the nuclear volume (Fig. 1), rarely lying in intimate contact with the nuclear envelope (Fig. 2). The nucleolar components, granular and fibrillar, are frequently disposed as a continuous network that constitutes the nucleolonema. Vacuolar nucleoli have fibrillar centers representing a condensation of the fibrillar component around clear nucleolar areas (Fig. 3). The cytoplasm of treated cells sometimes contains dense bodies, whose content becomes gradually clear, giving rise to numerous vacuoles (Fig.4). The subsequent confluence of these vacuoles leads to an extensive cytoplasmic lysis (Fig. 5). In the nucleus the presence of two nucleoli is a common phenomenon. In most cases the nucleoli exhibit intimate spatial relationships with nucleus periphery (Fig. 6), reflecting an intense transfer of ribosomal precursors to the cytoplasm. The nucleolus size increases, sometimes occupying almost the whole nuclear volume (Fig. 7). The nucleolar components become progressively more compact with a subsequent diminution of the vacuolar spaces and the rearrangement of the fibrillar component. From hypertrophied nucleolus, fibrillar microspherules are often released. These microspherules become free in the nucleus mass (Fig. 8) where they appear as a veritable mininucleoli constellation. This aspect resembles ribosomal gene amplification usually encountered in amphibian oocytes as well as in some polytenic and polyploid nuclei. After thyoacetamide administration, nuclear bodies with a variable complexity, existing sometimes even in untreated cells, become more frequent. The simplest of these bodies have a sheath of fibrous consistency and a granular matrix (Fig. 9), their appearance seems to be identical sometimes with that of fibrous microspherules that have been released from the nucleolus and which afterwards are surrounded by a fibrous sheath (Fig. 10). The abundant ribosomal precursor granules that are around these nuclear bodies reflect their possible role in the production of ribosomal precursors. The nuclear bodies, having close relationships with the nuclear envelope, manifest a tendency to pass into the cytoplasm (Fig. 11). These structures are of nucleolar origin and this supposition is based on the fact that sometimes the fibrillar component of the nucleolus gains a fibrous envelope (sheath) and is released from the nucleolus (Fig. 12).

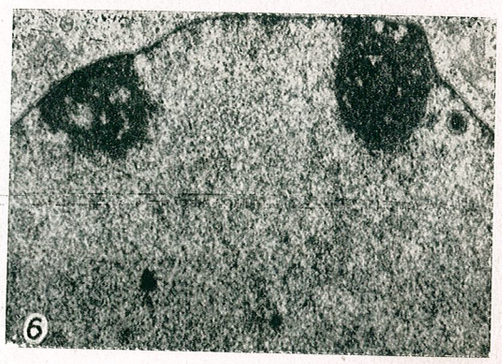
Figs. 1—3. — Ultrastructural aspects of untreated cells.
Figs. 4—12. — Ultrastructural aspects of treated cells.
Fig. 1. — A compact nucleolus from which a granular material is released. × 19,600.
Figs. 2, 3. — Vacuolar nucleoli with several fibrillar centers (arrows). × 14,700; × 39,200 respectively.
Figs. 4, 5. — Clear cytoplasmic areas from which the organelles have been destroyed. × 7,000; × 9,500 respectively.
Fig. 6. — A nucleus with two nucleoli in intimate contact with the nuclear envelope. × 19,600.

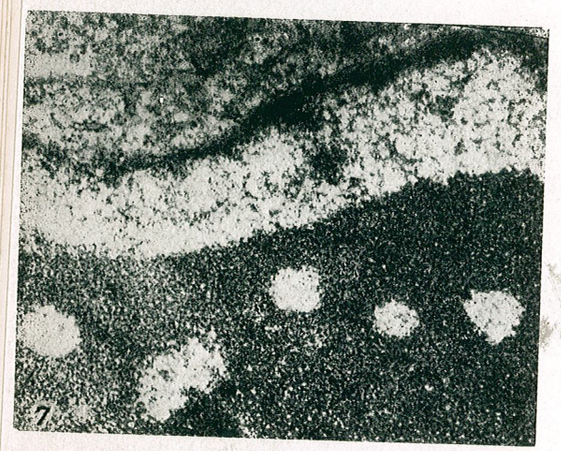


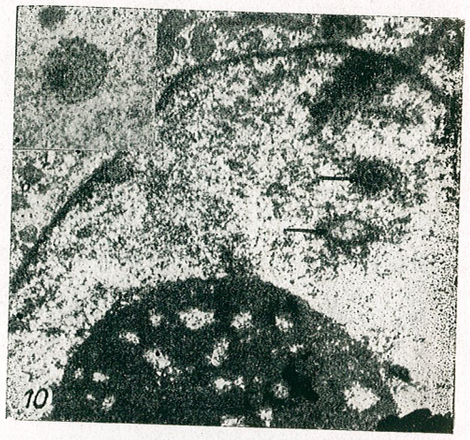


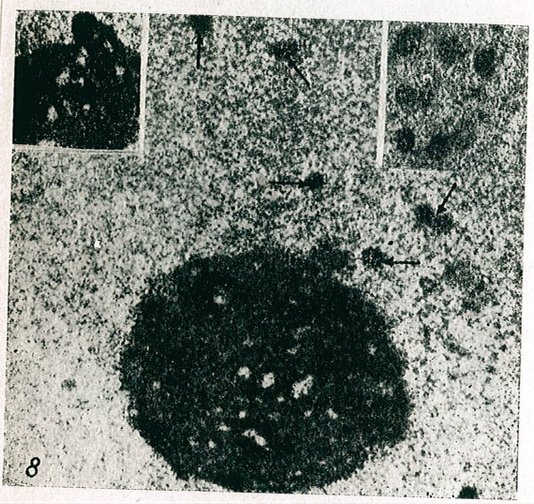


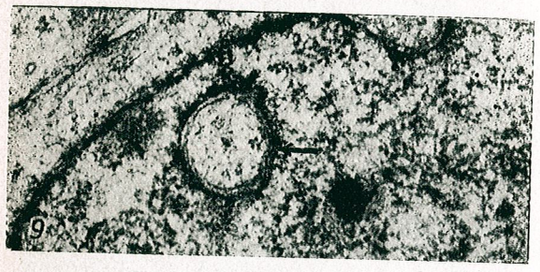


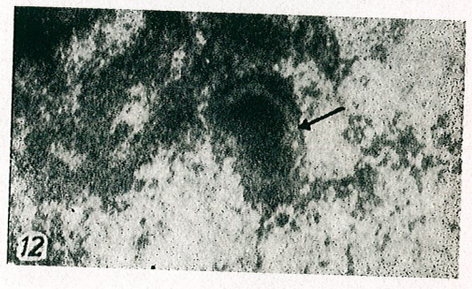












DISCUSSION

Thyoacetamide has an obvious effect on HEp2 line cells leading to a local lysis of cytoplasmic components. As a result of cytoplasmic ribosome quantitative diminution, the nucleolus, a veritable RNA deposit [3] becomes hypertrophied, a sign of a high rate of ribosomal precursors synthesis [9]. The intensification of nucleo-cytoplasmic exchanges is reflected by the high frequency of nucleolar bordering. The coalescence of fibrillar nucleolar components and their migration into the nucleoplasm have also been encountered in the liver cells [8]. The significance of this phenomenon is largely unknown but it might be related to the nucleolus hyperactivity and the cellular stress process. Nuclear bodies, whose functional role is largely unknown, are considered as markers of malignant cells [10]. As a result of thyoacetamide treatment the number of nuclear bodies increases parallelling the appearance of fibrillar microspherules inside the nucleolus. It was inferred that these fibrillar microspherules, veritable micronucleoli, are involved in the production of granular ribosomal precursors. Later, these micronucleoli are transformed into nuclear bodies which have no functional role any longer and, consequently, manifest the tendency to pass into the cytoplasm. These nuclear bodies may originate within the nucleolus *via* condensation of nucleolar fibrillar component and its surrounding by a fibrous sheath. Their role remains largely unknown.

The data presented here lead to the conclusion that thyoacetamide has similar effects both *in vivo* [1], [2], [6], [8] and *in vitro*.

CONCLUSIONS

1. In the cytoplasm of HEp-2 cells treated with thyoacetamide, a local lysis of cytoplasmic organelles occurs.

2. An obvious hypertrophy of nucleoli is encountered in such cells and these nucleoli have close spatial relationships with the nuclear envelope.

3. The fibrillar nucleolar component undergoes a notable reorganization appearing as microspherules which are released from the nucleoli, giving rise to a mininucleolar constellation within the nucleus. This phenomenon resembles ribosomal gene amplification that was encountered under natural conditions in some special types of cells.

Fig. 7. — Gigantic, dense and vacuolated nucleolus. × 68,000.
Fig. 8. — Dense nucleolus from which fibrillar micronucleoli (arrows) are released. × 14,200. Left inset-initial stadium of releasing. × 12,600 ; Right inset-several aggregated micronucleoli. × 12,600.
Fig. 9. — A simple nuclear body (arrow). × 68,600.
Fig. 10. — Two nuclear bodies (arrows). × 21,300. Inset — a nuclear body surrounded by a granular network. × 19,600.
Fig. 11. — Some nuclear bodies in intimate contact with the nuclear envelope. × 27,000.
Fig. 12. — The nuclear body (arrow) formed in the nucleolus migrates into the nucleoplasm. × 74,000.

4. The mininucleoli seem first to function in the production of ribosomal precursors; later, they seem to transform in nuclear bodies whose number increases after thyoacetamide treatment.

5. The nuclear bodies are sometimes organized even within the nucleolus.

REFERENCES

1. Busch, H., Smetana, K., 1970, *The nucleolus*, Academic Press, London, New York.
2. Busch, H., Muramatsu, M., Adams, H., Steele, W. J., Lian, M. C., Smetana, K., 1963, Exptl. Cell Res., suppl. 9, 150.
3. Busch, H., Byvoet, P., Smetana, K., 1963, Can. Res., **23**, *3*, 2043.
4. Chakrabartty, P. K., Schneider, W. C., 1978, Can. Res., **38**, *7*, 2043.
5. Kleinfeld, R. G., 1957, Can. Res., **17**, *10*.
6. Laisd, A. K., Arch. Biochem. and Biophysics, 1953, **27**.
7. Muramatsu, M., Busch, H., 1962, Can. Res., **29**, *9*, 1100.
8. Narayan, K., Shankar, Steele, W. J., Busch, H., 1966, Exptl. Cell Res., **43**, *2*, 423.
9. Olson, O. J. Mark, Hatchett, S., Allan, R., Hawkins, T. C., Busch, H., 1978, Can. Res., **38**, *10*, 3421.
10. Smith Helene S., Springer, E., Hackett, A. J., 1979, Can. Res., **39**, *2*, 332.
11. Toolan, H. W., 1954, Can. Res., **14**, 600.

Received February 19, 1980

University of Bucharest
Faculty of Biology
Bucharest, Aleea Portocalilor, 1—3

YOLK PLATELETS BREAKDOWN AND ACID PHOSPHATASE ACTIVITY IN EMBRYONIC DEVELOPMENT OF FISHES (BLASTULA AND GASTRULA STAGES)

BY

DRAGOȘ SCRIPCARIU, LOTUS MEȘTER and RADU MEȘTER

During early embryonic development of fishes *(Hypophthalmichthys molitrix)*, some platelets show a progressive degradation. The disintegration of yolk platelets occurs selectively and leads to the formation of a large amount of small and dense granular material. An acid phosphatase (beta glycerophosphate phosphatase) was also cytochemically identified at the periphery and in the heterogeneous structure of yolk platelets, which are in the process of degrading. Cytochemical data suggest the importance of acid phosphatase in the selective breakdown of yolk platelets during early embryonic development of fishes.

Several studies have shown that during differentiation a progressive degradation and disintegration of yolk platelets occurs, but the role played by enzymes in platelets breakdown is very little understood [2], [6], [8], [16]. Denis [1] identified a phosphoprotein phosphatase and a beta glycerophosphate phosphatase associated with the yolk platelets, in mature eggs and during the development of *Pleurodeles* embryos. Cytochemical demonstration of acid phosphatase participation in the process of yolk breakdown was later obtained on platelets of the Mexican axolotl embryos [6].

There are few data about possible factors involved in yolk platelets degradation during embryonic development of fishes. In *Salmo gairdneri*, no acid phosphatase activity could be demonstrated in unfertilized eggs, though the enzyme was identified in a later stage of development, associated with the degrading yolk platelets [13]. An acid phosphatase was cytochemically identified in the yolk syncytial layer from the alevin of trout, after hatching [14], [15].

Previous studies carried out in our laboratory have revealed cytochemically an acid phosphatase in growing and mature oocytes in some species of fishes, at the periphery of yolk platelets [12]. The enzyme was also demonstrated in the mature unfertilized and fertilized oocytes (after 20 min) of fish, in the vicinity of those platelets which are in the process of degrading [7].

The purpose of this study was to follow fish yolk platelets breakdown during early stages of embryonic development, in correlation with the presence of acid phosphatase at their level.

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, Nº 2, P. 131—135, BUCAREST, 1981

MATERIALS AND METHODS

Our experiments were carried out on *Hypophthalmichthys molitrix* (silver carp) acquired from a fish hatchery (Nucet Research station).

Blastula and gastrula stages were removed from their jelly coats and were fixed in 2.5% glutaraldehyde solution buffered to pH 7.4 with 0.1 M cacodylate, containing 4% sucrose, for 30 min at cold. Afterwards, the pieces were washed 4—5 times with cacodylate buffer 0.1 M, pH 7.4, with sucrose (4%), at cold. The histochemical reaction for acid phosphatase was performed by incubating the pieces in a modified Gomori incubation medium at pH 5.0, as described by Pearce [9]. For controls, tissues were processed in the same incubation medium without substrate (beta glycerophosphate). The incubation was carried out at 37°C, for two hours. After incubation, the tissues were rinsed several times with cacodylate buffer 0.1 M, pH 7.4 and postfixed in 1% osmium tetraoxide, buffered with 0.1 M cacodylate buffer pH 7.4, for 60 minutes at 4°C. The tissues were dehydrated through a graded series of ethanol to propylene oxide and embedded in Epon.

For electron microscopy, silver-gold sections were cut on a LKB ultratome, stained with a lead citrate solution (4%) and examined with a Philips 201 electron microscope.

RESULTS

1. *Yolk platelets degradation.* Yolk platelets represent one of the most prominent components in the cytoplasm of mature unfertilized eggs. They also appear as major constituents of cells, during early developmental stages. The majority of yolk platelets apparently have lost their membranes and appear ultrastructurally as a population of large and dense structures with a heterogeneous content.

In the blastoderm cells, some of these large yolk platelets undergo a process of progressive breakdown. Electron microscopy shows that these platelets appear disrupted structurally in smaller pieces (Pl. I, Figs. A and C). This progressive degradation of platelets takes place in different ways : by disruption of the central core, by the formation of smaller units and by lysis, which may occur both from the periphery and from the interior of yolk platelets. However, in the stages of blastula of fish development, only some platelets are in the process of degrading. Probably, the degradation of yolk platelets appears polarized or limited to some regions of embryos or of some cells.

Yolk platelets breakdown in gastrula cells stage of fish shows a more heterogeneous structural aspect. As can be seen in Figs. A-D (Pl. II), yolk platelets are broken down in many smaller units. They are characterized by a heterogeneous nature of their content, without a visible limiting membrane. Ultrastructural observations also shows different degrees of degradation of platelets. The electron microscopic images suggest that the breakdown of platelets is a complex process, which involves a weakening of the central structure, the formation of smaller subunits and a subsequent dispersion of the yolk granular material in the cytoplasm (Pl. II,

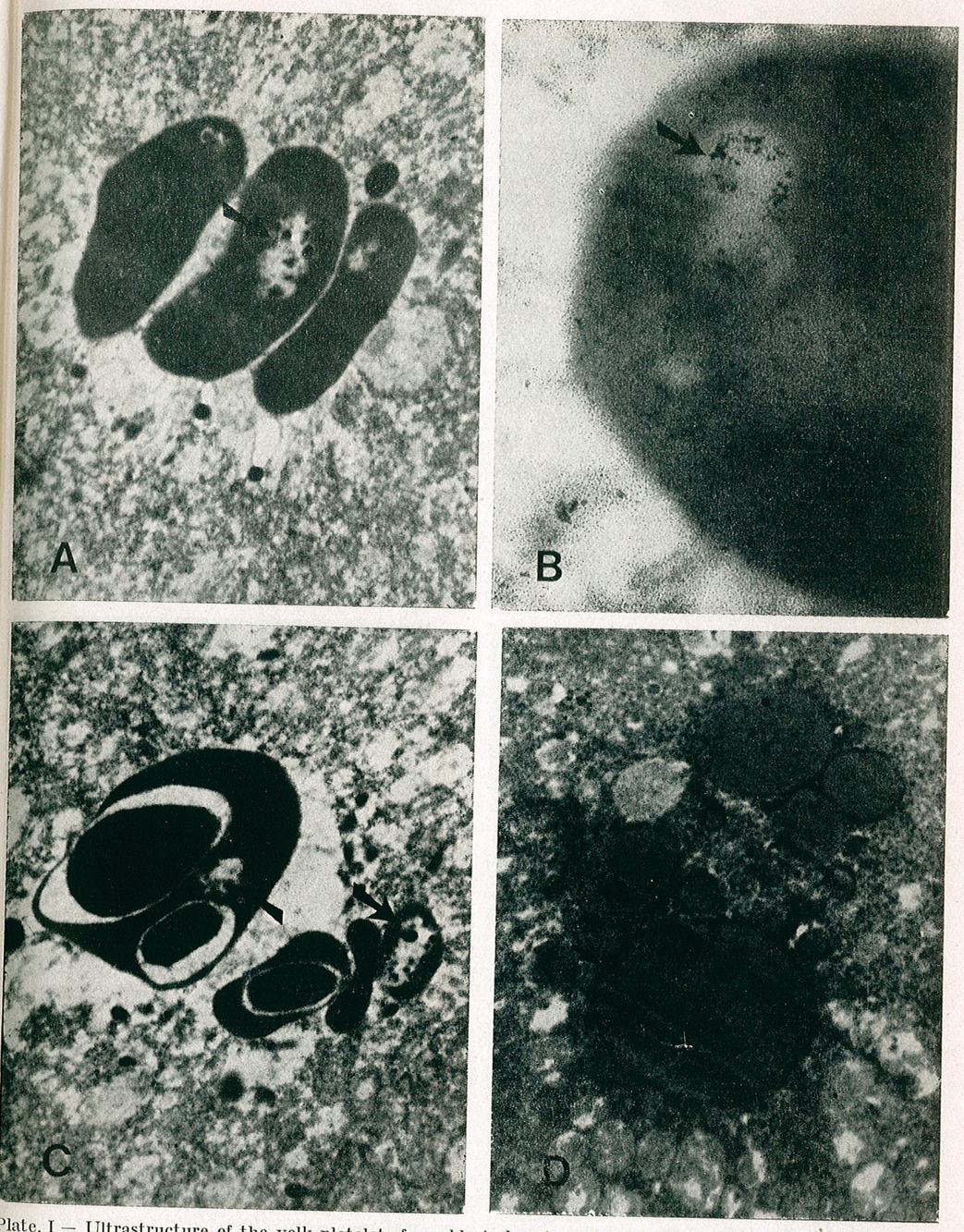


Plate. I — Ultrastructure of the yolk platelets from blastodermic cells of developing fish embryos.
A) Yolk platelet in an early stage of degradation. The enzymic reaction product of acid phosphatase is visible in the solubilization areas (see arrow). 10.000×.
B) A yolk platelet at a higher magnification (30.000×). Note the presence of the reaction product of acid phosphatase within its interior structure.
C) Breakdown of yolk platelet illustrating the burst of its structure with formation of heterogeneous smaller subunits. Acid phosphatase is visible in the solubilization areas (see arrows). 10.000×.
D) Yolk platelets with a positive reaction for acid phosphatase at their periphery. 7500×.

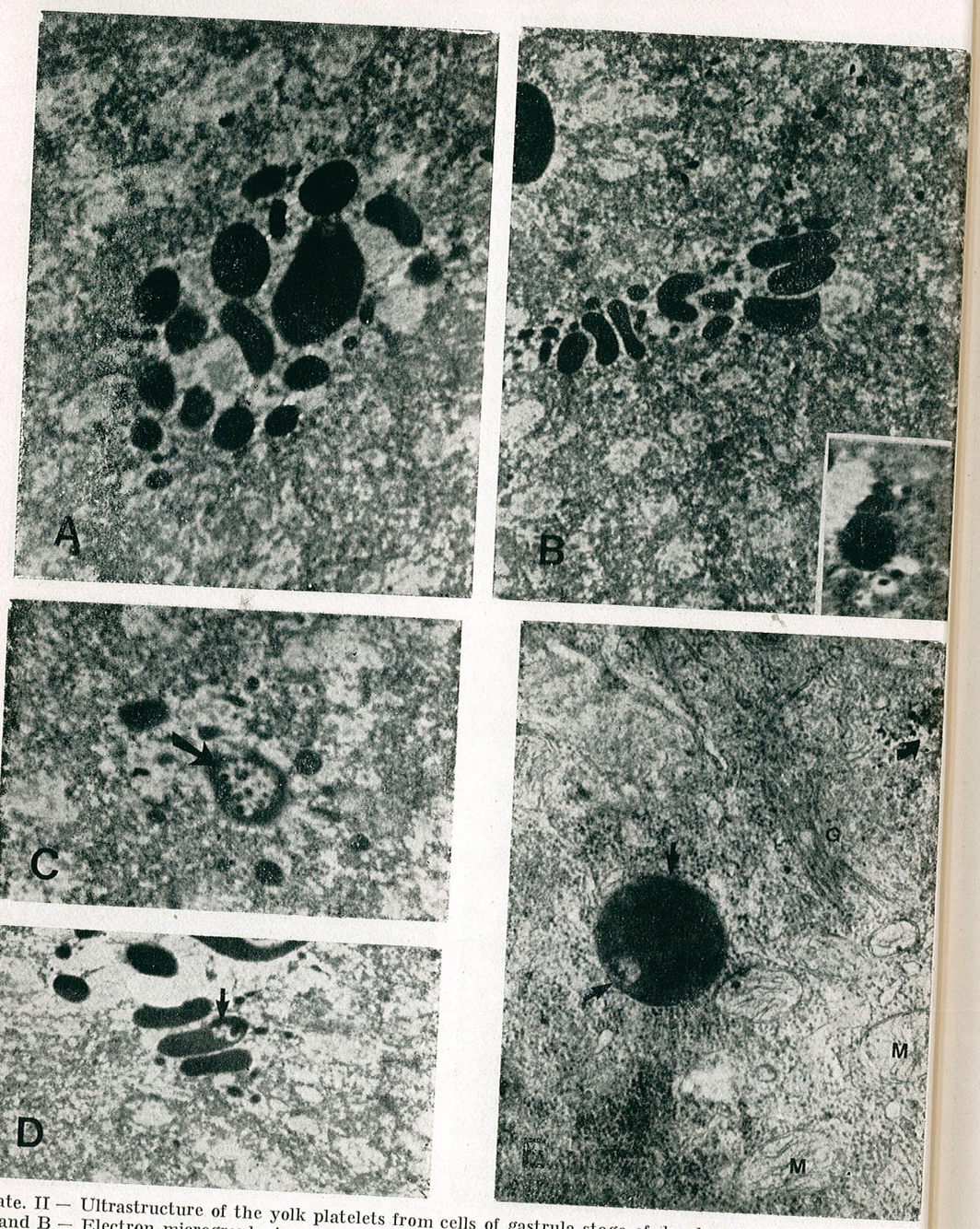


Plate. II — Ultrastructure of the yolk platelets from cells of gastrula stage of developing fish embryos.
A and B — Electron micrographs images illustrating the degradation of yolk platelets structure. In some of these small units, acid phosphatase was identified (Fig. B, higher magnification). 10.000×.
C and D — Small degraded platelets surrounded by numerous granular bodies with positive reaction for acid phosphatase (arrows). 12.500×.
E) Electron micrograph image showing Golgi apparatus with its secretory vesicles and a small degraded platelet (granular material). In both structures, acid phosphatase was identified (see arrows). 12.500×.

Figs. C and D). Sometimes this yolk granular material appears visible with a limiting membrane, which probably represents the final product of yolk platelets degradation.

2. *Ultrastructural localization of acid phosphatase activity.* In early stages of differentiation, yolk reserve utilization depends on many factors. Since acid phosphatase activity was identified at the periphery of some platelets in mature unfertilized and fertilized fish eggs, we followed the association of this enzyme with yolk platelets breakdown during early stages of fish development.

In the blastoderm cells, the reaction product of enzymatic activity was identified at the level of yolk platelets which are in the process of degrading. The localization of acid phosphatase on these structures appears with a heterogeneous distribution. The enzymic reaction product was identified at the periphery of some yolk platelets (Pl. I, Fig. D) or in the central part of the structure (Pl. I, Fig. A and C; at a higher magnification Fig. B).

Acid phosphatase is also present at the level of smaller subunits of platelets (which are in the process of degrading), described in gastrula stage of fish. Because of their strong osmiophily, the enzymic reaction product of acid phosphatase appears sometimes hardly distinguishable. Electron microscopic images show a positive reaction in the interior of subunits yolk platelets (Pl. II, Figs. A and C, arrows) and at their periphery (Pl. II, Fig. D, arrow). At a higher magnification, acid phosphatase can be observed cytochemically, both at the periphery and in the interior of a small yolk granulary material dispersed in the cytoplasm (Pl. II, Fig. B).

In some gastrula stage cells the structural elements of the Golgi apparatus were detectable, associated with a population of vesicles which present a positive reaction for acid phosphatase (Pl. II, Fig. E, arrow). In the image, a yolk platelets granule is also visible, with acid phosphatase activity distinguishable at its periphery. This granule is delimited by a membranous envelope.

DISCUSSION

During early developmental stages, yolk platelets furnish the specific material for morphogenesis and organogenesis. Although it is evident that the disappearance of yolk platelets is related to the occurrence of cell differentiation, the nature of this relationship is not well understood.

Structural organization of yolk platelets in fish appears to be different form those described in amphibians [3], [6], [10]. Our data show that yolk platelets appear as ovoid structures with an amorphous content. This morphological aspect of yolk platelets structure is in agreement with the observations obtained on trout yolk [13]. The earliest changes in the platelets during development consist in the breakdown of the large structure in small pieces with polymorphous aspect. This process occurs in different parts of the platelets and leads to the formation of large amounts of dense granular material (or subunits) of different sizes.

There are some general cytological data concerning the amphibian yolk platelets degradation [3], [6], [16]. Yolk platelets of developing axolotl embryos degrade by membranous unraveling or delamination at their periphery [6]. The breakdown process begins at the periphery by the disappearance of the original superficial granular zone, and later by the degradation of the main crystalline part of the yolk platelets.

Yolk platelets breakdown in the early stages of fish embryonic development is not a general phenomenon. Probably, the individual yolk platelet is solubilized by a coordinative process, with the participation of unknown cellular factors of control. Most yolk platelets remain unaltered in the cells until the time of hatching. The high rate of their solubilization takes place during the growth phase after hatching and finishes with the disappearance of yolk vesicles.

Our cytochemical data revealed the presence of an acid phosphatase activity in blastula and gastrula stages of fish embryonic development, associated with yolk platelets utilization.

Different opinions exist regarding the intracellular location of acid phosphatase activity, in the oocytes and during the first stages of development of fish and amphibians. In some cases, acid phosphatase was demonstrated in growing *Rana pipiens* oocytes [4] and growing fish oocytes [12]. Steiner and Hanocq [11] failed to identify an acid phosphatase activity at the level of yolk platelets in mature *Xenopus laevis* oocytes, but a positive reaction has been shown at the level of Golgi apparatus. In fish, acid phosphatase was identified in the plasma membrane, in the cortical alveoli and the cytoplasmic matrix of *Carassius* oocytes [5], or in the yolk syncytial layer in the alevin of trout after hatching, and only occasionally in the first stages of differentiation [13]—[15].

The presence of acid phosphatase in the first stages of development (gastrula) was demonstrated on amphibia [6]. In contrast with the results obtained by us on fish, yolk platelets breakdown in early stages of amphibian development appear only at their periphery. The presence of acid phosphatase in the inner structure of amphibian platelets was identified only occasionally.

Our present data, in correlation with previous studies [7], [12], suggest that phosphomonoesterasic activity is present in growing fish oocytes and also in the first stages of fish embryonic development, in correlation with the utilization of yolk. Furthermore, acid phosphatase activity continues with different rates in all stages of fish embryonic development.

REFERENCES

1. Denis H., 1964, J. Exper. Embryol. Morphol., **12**, 197.
2. Dingle J. T., 1977, in *Lysosomes*, North Holland Pub. Comp., Amsterdam.
3. Karasaki S., 1963, J. Ultrastruct., Res., **9**, 225.
4. Kessel R. G., Decker D. S., 1972, J. microscopie, **14**, 169.
5. Kudo S., 1976, Annot. zool. Japon., **49**, 105.
6. Lemanski L. F., Aldoroty R., 1977, J. morphol., **153**, 419.
7. Meşter R., Scripcariu D., Meşter L., 1980, St. cerc.-biol—Zool., **32**, 23.

8. Pasteels J. J., 1973, in *Lysosomes in Biology and Pathology*, ed. Dingle J. T., vol. III, North Holland Pub. Comp., Amsterdam.
9. Pearce A. G. E., 1968, in *Histochemistry Theoretical and Applied*, vol. I, Little Brown, Boston.
10. Ohlendorf D. H., Wren R. F., Banaszok L. J., 1978, Nature, **272**, 28.
11. Steiner G., Hanocq J., 1979, Biol. Cellulaire, **34**, 247.
12. Varo I. M., Meşter R., Scripcariu D., Topîrceanu F., 1979, Rev. Roum. Biol.—Biol. Anim., **24**, 117.
13. Vernier J. M., Sire M. F., 1977, Biol. Cellulaire, **29**, 99.
14. Walzer J. M., Schonenberger N., 1979, Cell Tissue Res., **196**, 59.
15. Walzer J. M., Schonenberger N., 1979, Cell Tissue Res., **196**, 75.
17. Williams J., 1967, in *The Biochemistry of Animal Development*, ed. Weber R., vol. II, Academic Press, New York.

Received April 21, 1980

Faculty of Biology
Laboratory of Cell Biology
76201, Bucharest 35,
Bucharest, Splaiul Independenţei, 91—95

CEREBRAL VESICLE ALOTRANSPLANT TO CHICK EMBRYO
I. CHANGES IN THE HEAD AND THE BRAIN STEM OF THE HOST

EMILIA GHIDUȘ, OLGA CILIEVICI, Z. KNEZEVIĆ

Serial sections stained by Nissl's method, hematoxylin-eosine and Spielmeyer's method were used for the examination of a chicken encephalon, which in the stage of 13 somites, after decapitation, was grafted on a corresponding fragment collected in the same way from another embryo of the same age. The chicken developed with two complete beaks. Making a comparison with a normal encephalon it was clarified the place and way in which the two components (the donor and the receiver) were sutured. The host brain stem was analysed in detail and some aspects (hypertrophied ectopic ganglion, broken and displaced formations, conjunctive tissue bridges, etc.) that might, at least partially, contribute in the mechanism of induction of malformations were discussed.

Embryonary transplants supply valuable data both for understanding some important problems of biology (morphogenetic induction, determination, transdetermination, differentiation, development, etc.) and for clarifying the mechanism by which various malformations are produced.

The literature concerning cerebral transplants presents mainly the external structures which they influence during their development.

The purpose of the present work was to obtain cerebral vesicles alografts and to analyse on serial microscopic sections the way in which the two cerebral components (from the donor and the host) evoluate.

MATERIAL AND METHOD

The work was carried out on Rhode Island hen eggs. At 36 hrs of incubation, windows 10/10 mm in size were cut in the calcareous egg shell just above the embryo. The latter were selected to have the same age and to be in the stage of 13 somites and the decapitation was then performed (which means more than it is commonly understood by decapitation in Sherrington's terms) on a stereomicroscope using a pair of scissors for iridectomy. The portion removed from one embryo was aspired with a micropipette and transplanted to another embryo from which, after decapitation, the corresponding fragment was removed. Survival of the embryos was 30% of which some showed various malformations. Sacrification was performed on the 21st day of incubation at the same time with the controls.

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, N° 2, P. 137–141, BUCAREST, 1981

In the present work we present an analysis of the encephalon of a cerebral alografted chicken presenting a congenital malformation : the presence of two complete beaks. The analysis is made by comparison with the encephalon of a normal chicken (Figs. 1, 2, 3).

Each encephalon was cross-sectioned into 3 fragments (prosencephalon, mesencephalon and rhombencephalon + cerebellum) which were imbedded in celloidin. The serial sections were stained by three methods : Nissl, hematoxylin-eosine and Spielmeyer).

RESULTS AND DISCUSSIONS

Examination on serial sections of the alografted encephalon allowed us to specify, by comparison with the normal encephalon, the site of suture of the two cerebral components and to analyse their structure. The grafted fragment which included the prosencephalon and portions of mesencephalon and cerebellum, suffered by aspiration and grafting, lateral compression which determined shortening and asymmetry of the two halves of the brain and, important torsion due to which at the site of suture to the host, the optic tuberculum of one side assumed an abnormal position. The site of suture between the two components was filled with a lax tissue due to the proliferation of brain covers.

The main aspects noticed on the serial sections in the brain stem of the host by comparison with those noticed in the normal chicken, are presented below starting from the level of hypoglossus nerve nuclei to the red nucleus.

Sections containing the inferior half of the hypoglossus nerve nuclei show that the latter contain less neurons than in the normal chicken and that some of these neurons have a greater amount of tigroid bodies. In the normal chicken the cerebellum appears at this level ; in the grafted one the cerebellum may be seen only at the level of the upper poles of the hypoglossus nerve nuclei and is dorso-laterally pushed drawing with it the corresponding choroid plexuses. Here may also be seen the lower pole of the dorsal nucleus of the vagus nerve containing neurons, too, unequally loaded with the ergastoplasm. Along a short distance the ependymary channel is broken and by proliferation of the displaced choroid plexuses a lax tissue was formed, with thin bridges connecting the right half of the bulb to the left one. The nuclei of the reticulate formation at this level (the raphys nucleus and magnocellularis ones) contain more neurons than in the control chicken. Somewhat more cranially, when the dorsal nucleus of the vagus is large, the displaced cerebellum appears broken and torsioned and the covers of the brain stem (especially on the cerebellum side) are excessively thickened (Figs. 5, 6). Displacement of the cerebellum took along the corresponding half of the brain stem and that is why, starting from the level of the commissura infima and of the glossopharingeal nerve nuclei, the images of the two halves become asymmetrical. At this level, besides the well-developed tissular bridges, the sections also show a tissular formation including a ganglion which will later adhere to the lateral wall of the encephalon in the cochlear nuclei area (Figs. 7, 8). In this last area some things are noteworthy (Fig. 9) :

Fig. 1. — Chicken with 2 beaks.

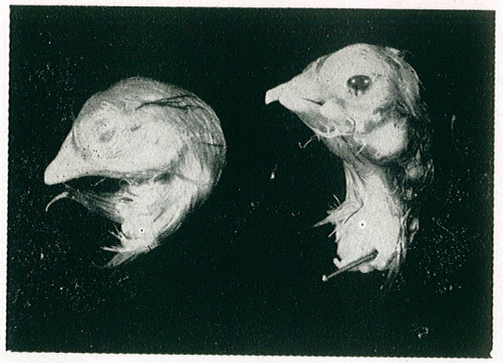


Fig. 2. — Normal chicken and chicken with 2 beaks (on the 21st day of incubation).

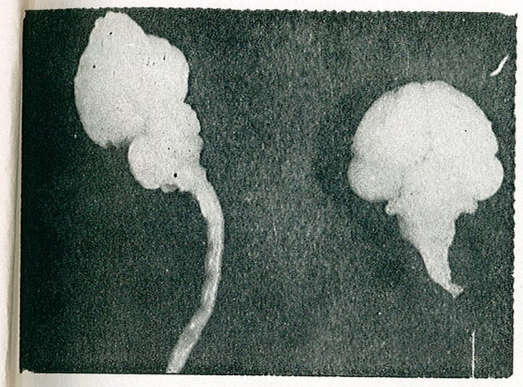


Fig. 3. — Grafted encephalon beside normal encephalon.

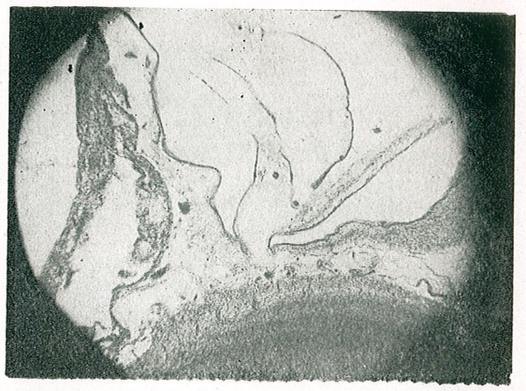


Fig. 4. — Site of suture of two cerebral components ; on the left, the host ; below, the optic tuberculum from the donor ; above, on the right, conjunctive bridges.

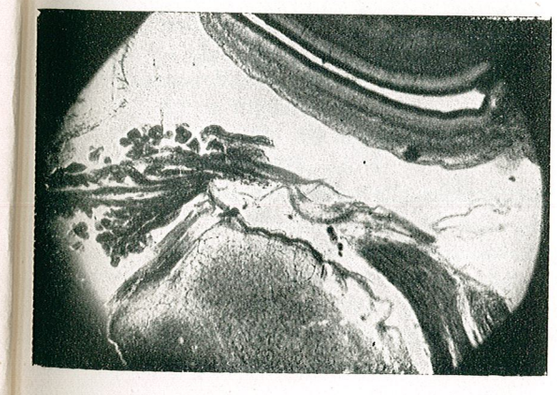


Fig. 5. — Section through the brain stem at the level of the glossopharingeal nerve nucleus. Broken choroid plexuses, the conjunctive bridge at the level of commissura infima, the cerebellum moved and the tissular formation including the ganglion (on the right) can be noticed.

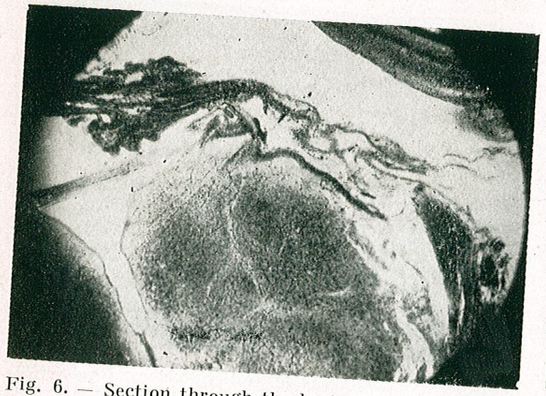


Fig. 6. — Section through the brain stem at the level of the vagus motorius dorsalis nucleus. The same elements as in Fig. 5.

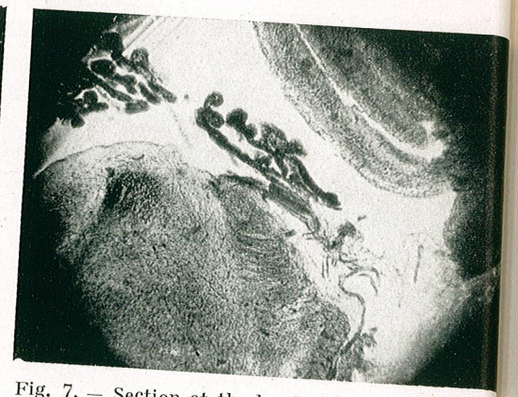


Fig. 7. — Section at the level of the cochlearis an laris nucleus ; the ganglion can be seen on the rig

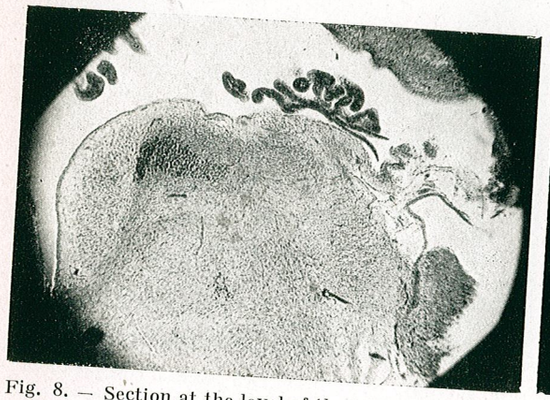


Fig. 8. — Section at the level of the cochlearis magnocellularis nucleus. The ganglion adhering to the lateral wall can be seen.

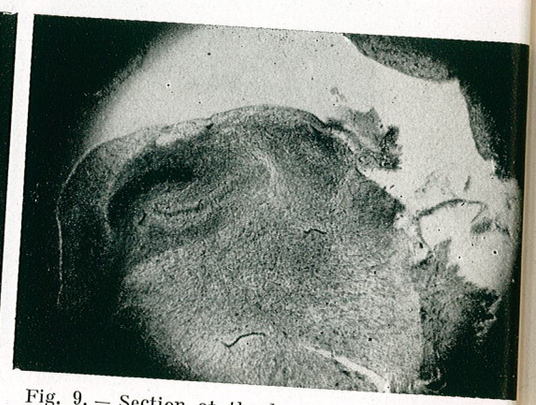


Fig. 9. — Section at the level of the trapezoid bo equivalent. Large neurons of the vestibularis nuc and of the trigeminus motor nucleus and the fibr going towards the ganglion can be noticed.

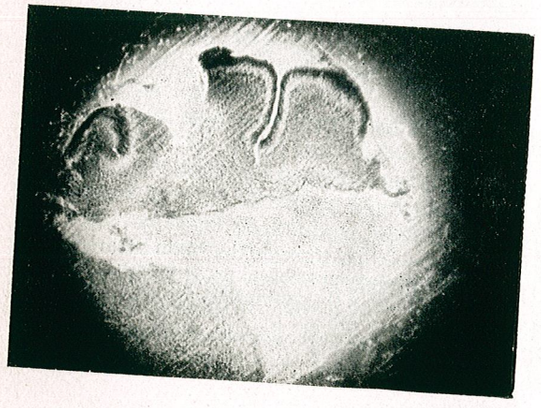


Fig. 10. — Cerebellum fragment from the host.

the cochlearis magnocellularis nucleus is more distal in the grafted chicken than in the normal one, from the IVth ventricle walls and the cochlearis linearis nucleus contains less well-stained neurons ; commissura cochlearis was broken by the movement of the cerebellum which determined an alteration in the form of the IVth ventricle though the newly formed connecting bridges are present up to the level equivalent to the trapezoid body. At this level vestibular nuclei and the motor nucleus of the trigeminus may be noticed, with big neurons ; facial nerve nuclei, and adhering to the lateral wall the ganglion which reaches its maximum dimensions may also be noticed. It is possible that this ectopic and hypertrophied ganglion be implied in the morphogenetic process of inducing malformation causing an additional beak. In the brain stem of the host it may also be seen : the nucleus of the 6th pair of cranial nerves and the zone in which the cerebellum was cut off from the brain stem and did not allow consolidation of the cerebellar pedunculi. The cerebellum is larger, shows portions of cerebellaris nuclei but it is torsioned and has necrotic areas. A small cerebellum fragment also occurs on the opposite side (Fig. 10). Further, one can also see the trigeminus sensible mean root nucleus, an area of large neurons of the reticulate formation and, on few sections, the isthmo-optical nucleus, on one side only.

In the control, up to the isthmo-optical nucleus, the sections show clearly other formations as well : the nucleus of the 4th pair of cranial nerves, the optic tuberculum, the lemnisci lateralis ventralis nucleus, the linearis caudalis nucleus in the reticulate formation. The isthmo-optical nucleus can be seen at the same level with the semilunaris nucleus and the tegmentalis nuclei and, on few sections, with the lower poles of the oculomotor nerve nucleus, too. The red nucleus becomes visible in the mesencephalic reticulate formation at the level of the accessory nucleus of the 3rd cranial nerve pair and of the mesencephalic nuclei.

Our study suggests that the zone delimited by the isthmo-opticus nucleus and the red nucleus can be followed neither in the brain stem of the host nor in the grafted fragment (though it may not be completely missing, but only altered so much as to become unrecognizable).

The literature shows, on the basis of the data obtained by embryonary grafts and extirpations performed at the cerebral vesicle level, that a certain neuron territory determines the appearance of the bone tissue. It was thus possible to show the role played by the whole encephalon and its various section (prosencephalon, mesencephalon, and rhombencephalon) and even by the spinal cord in the morphogenetic induction that causes the development of the neurocranium [10], [5], [11]—[17]. The mesencephalon, which in our transplant suffered mostly, does not seem to play a significant role in the morphogenetic induction of the head bones but only to supplement the induction of the prosencephalon and rhombencephalon [17].

We may say that the grafted fragment, though altered during sectioning and grafting, played its role of a morphogenetic inducer in the viscero- and neurocranium development, by its components and in cooperation with the brain stem of the host.

The malformation on the beak which is, as we have shown, one of the malformations encountered in the cerebral alograft lot, could be re-

garded as a consequence of the alterations produced in various areas by the intervention of the experimenter. And these are not few in number. Schowing [12] shows that the upper beak develops from the frontal bud elements. Therefore, in our experiment, it is possible that part of the frontal bud may have moved to another area or that part of the graft forced into altered conditions induced a supplementary frontal bud. It may equally happen that new elements such as a Gasser ganglion, ectopic and hypertrophied, broken and displaced choroid plexuses, fragments of cerebellum with lesions, newly formed conjunctive bridges, etc, contributed to the morphogenetic induction of the elements from which the second beak was made up.

The intimate mechanism of production of this malformation still remains to be clarified even though genetically it may also be regarded as a case of genic overdosage and perturbations in the genic control in areas in which the genetic material underwent changes.

CONCLUSIONS

Examination on serial sections of the chicken encephalon with cerebral graft and congenital malformations as well as the presence of two well-formed beaks by comparison with the control chicken, allowed the following conclusions:

1. The encephalon of the alografted chicken took the prosencephalon and parts of the mesencephalon and the cerebellum, from the donor, and the brain stem up to the level of the isthmo-opticus nucleus and an important part of the cerebellum, from the host.
2. The place and way in which the two cerebral components joined could be clarified.
3. The mesencephalon shows the greatest formation deficiencies as decapitation has been performed at this level with both embryos.
4. The alograft made in the stage of 13 somites allowed the newly formed encephalon to carry out its morphogenetic action on the skeleton (in case this action had not been carried out before).
5. The alterations produced at various levels of the encephalon during the operation of grafting, i.e., displacements, compressions, breakings and torsionings of structures, as well as the special development of some formations (conjunctive bridges, fill-up lax tissues and especially a large ectopic ganglion) may contribute in producing the malformation.

The transplants were performed within the Department of Radiobiology, Boris Kidrić Institute of Nuclear Sciences, Vinća, Beograd, Yugoslavia. The embedding in celloidin and processing of sections were carried out in Bucharest, at the Neurology Institute, Depart. of Pathologic Anatomy.

REFERENCES

1. Brachet J., Denis H., 1963, Nature, **198**, 205–206.
2. Ghiduș Emilia, 1974, Doctorial thesis, Univ. București.
3. Hagen E., Wechsler W., Zilliken P., 1967, *Morphological and Biochemical Aspects of Cytodifferentiation*, S. Karger, Basel, New York.

4. Hamburger V., Hamilton H. L., 1951, J. Morphol., **88**, 49–92.
5. Huber W., 1957, Arch. Anat. micr. Morph. exp., **46**, 325–405.
6. Jungherr E. L., 1945, J. Comp. Neurol., **82**, 55–75.
7. Jungherr E. L., 1969, *The neuroanatomy of the domestic fowl Gallus domesticus*, Avian Diseases, Univ. of Massaschusettes Amherst. Special Issue.
8. Kappers A., 1947, *Anatomie comparée du système nerveux, particulièrement de la cellule des mammifères et de l'homme*, Paris.
9. Petrovicky P., 1966, Folia Morphologica (Prague), **4**, 334–345.
10. Rogers K. T., 1964, Am. J. Anat., **115**, *3*, 487–508.
11. Schowing J., 1964, C. R. Acad. Sci. Paris, **259**, 2030–2033.
12. Schowing J., 1965, J. Embryol. exp. Morphol., **14**, 255–263.
13. Schowing J., 1968, J. Embryol. exp. Morphol., **19**, *1*, 9–22.
14. Schowing J., 1968, J. Embryol. exp. Morphol., **19**, *1*, 23–32.
15. Schowing J., 1968, J. Embryol. exp. Morphol., **19**, *1*, 83–93.
16. Schowing J., Rabadey, 1971, C. R. hebdomadaires des Séances de l'Acad. de Science, **272**, ser. D, *12*, 23–25.
17. Wolff E., 1969, *Les interactions tissulaires au cours de l'organogenèse.* Dunod, Paris.

Received January 7, 1980

Faculty of Medicine, Lab. of Biology and Genetics
Bucharest, Romania
Inst. of Nuclear Sciences, Dep. of Radiobiology, Vinća,
Beograd, Yugoslavia

AGE-DEPENDENT CHANGES IN THE GLYCOGEN CONTENT IN SOME TISSUES OF *MYTILUS GALLOPROVINCIALIS* (L) OF THE BLACK SEA

BY

NINA ŞILDAN, IOSIF MADAR and EUGEN A. PORA

The glycogen content of the hepatopancreas, the non-gonadal mantle tissues and the posterior adductor muscle of *Mytilus galloprovincialis* was followed in seven size groups, corresponding to various age groups.

It was established that in summer the tissues of mussels contain large amounts of glycogen, the greatest carbohydrate reserve being found in the mantle and the lowest in the muscle. On the basis of energy storing capacity of the examined tissues, two critical periods, corresponding to body lengthes of 2.2 cm and 3.2—4.2 cm in the postlarval development of the mussels, were noticed.

In well-fed bivalve molluscs the amount of glycogen stores may be very large, even up to 60 percent of the dry body weight in some species. Significant changes in the glycogen content are occurring in hibernation and starvation [6], [7], [12]. The common sea mussel, *Mytilus edulis* (*L*), contains large amounts of glycogen subjected to intense seasonal and reproductive variations [3], [5], [13], [14].

Our recent works [4], [9], [10], [11] provide a strong support to the hypothesis that in *Mytilus galloprovincialis* beside a cellular metabolic regulation there exists a hormonal regulation of the carbohydrate metabolism. On the other hand, our data show that in *Mytilus galloprovincialis* the glucose concentration in the haemolymph is relatively constant within different age groups and that the level of circulating glucose is decreasing parallelly with the ageing of the mussels [8].

These findings led us to the present work, in which the glycogen content in three main carbohydrate storing tissues of *Mytilus galloprovincialis* in seven size groups, corresponding to different age groups, was examined.

MATERIAL AND METHODS

Mussels of various sizes were collected between 15—25 August 1975, from the low water littoral zone of the Black Sea at Agigea (I.R.C.M.—Constanţa). After cleaning the shells, the mussels were selected and kept for 3 days, prior to the experiments, in aquariums in sand filtered, aerated, natural sea water with a total salinity of 15—16‰ at a temperature of 22°C. For the experiments 7 size groups (respectively age groups) of animals were used as follows : group I = 1.7 cm ; group II = 2.2 cm ; group

III = 3.2 cm; group IV = 4.2 cm; group V = 5.2 cm; group VI = 6.2 cm and group VII = 8.0 cm.

On the day of experiment, the mussels were removed, at intervals, from the aquariums and opened by cutting the adductor muscles. After blotting the interpallial water by filter paper, small pieces of the hepatopancreas, mantle and posterior adductor muscle were quickly removed. The mantle pieces for glycogen analysis were excised from the non-gonadal tissues avoiding at the same time the muscular mantle border. The glycogen determinations were made from 20—30 mg fresh tissue according to R. Montgomery [12].

The data, expressed as mg glycogen/100 g wet tissue, were calculated statistically according to Student's t test, P values of 0.05, or less, being considered statistically significant.

RESULTS AND DISCUSSIONS

The age groups of mussels used in these experiments seem to be well delineated, the size differences between every two neighbouring groups being highly significant (figure 1).

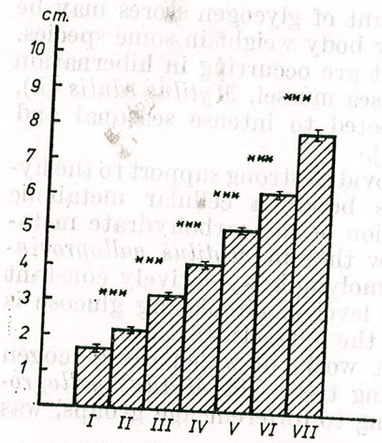
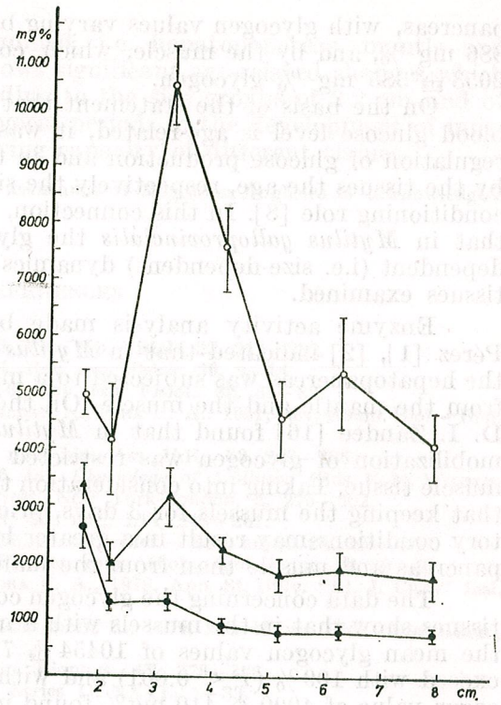


Fig. 1. — Mean body sizes (length in cm) of the seven experimental groups of mussels used for glycogen content analysis. I = 1.7 cm ($n = 10$); II = 2.2 cm ($n = 11$); III = 3.2 cm ($n = 12$); IV = 4.2 cm ($n = 10$); V = 5.2 cm ($n = 11$); VI = 6.2 cm ($n = 11$); VII = 8.0 cm ($n = 10$). ***) $P < 0.001$; n = number of animals.

The absolute mean values ± SE of the glycogen content in the hepatopancreas, the mantle tissues and the adductor muscle are presented in figure 2, and the percentage modifications as compared to the values obtained in group I (1.7 cm) are given in figure 3.

The data summarized in figure 2 show that in *Mytilus galloprovincialis* the carbohydrate reserves, in late summer, are noticeably high and comparable with the values reported for *Mytilus edulis* by other investigators [3], [5], [14]—[16]. At the same time, one can observe a characteristic tissue distribution of the glycogen stores which remain essentially the same throughout the life span of the mussels. Thus, the mantle contains the highest carbohydrate amounts, ranging from 4121 ± 604 to 10454 ± 719 mg glycogen per 100 g fresh tissue, followed by the hepato-

Fig. 2. — Changes in the glycogen content of the hepatopancreas (▲), the posterior adductor muscle (●) and the mantle (○) of *Mytilus galloprovincialis* depending on the body size.



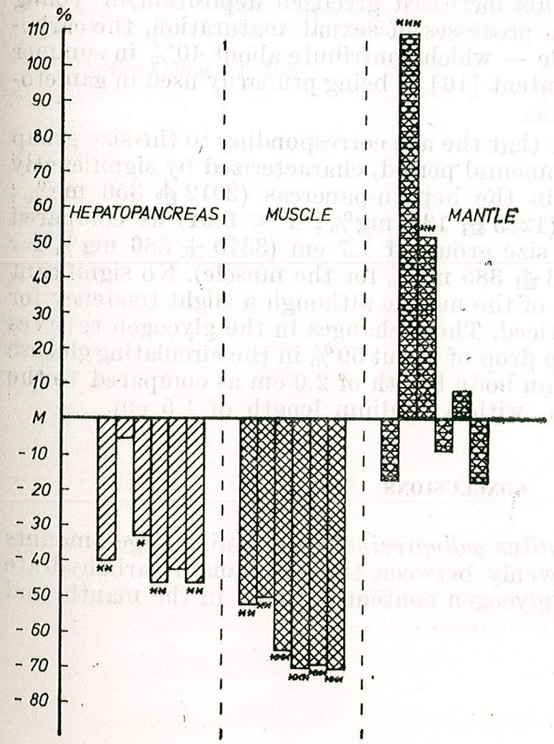


Fig. 3. — Percentage variations of the glycogen content in the hepatopancreas, the muscle and the mantle of *Mytilus galloprovincialis* as compared to the values obtained for group I (1.7 cm) *) $P \leq 0.05$; **) $P \leq 0.02$ and $P \leq 0.01$; ***) $P \leq 0.001$.

pancreas, with glycogen values varying between 1815 ± 310 and 3370 ± 386 mg %, and by the muscle, which contains between 796 ± 109 and 2653 ± 385 mg % glycogen.

On the basis of the statement that in *Mytilus galloprovincialis* the blood glucose level is age-related, it was assumed that in the hormonal regulation of glucose production and in the *in vivo* glucose consumption by the tissues the age, respectively the size, of mussels has an important conditioning role [8]. In this connection, from the present data it results that in *Mytilus galloprovincialis* the glycogen reserves exhibit an age-dependent (i.e. size-dependent) dynamics in all the carbohydrate storing tissues examined.

Enzyme activity analysis made by M. Alemany and M. Rosell-Pérez [1], [2] indicated that in *Mytilus edulis* the glucidic energy from the hepatopancreas was subjected to a more rapid mobilization than that from the mantle and the muscle. On the other hand, A. de Zwaan and D. I. Zandee [16] found that in *Mytilus edulis* during anaerobiosis the mobilization of glycogen was restricted to the hepatopancreas and the muscle tissue. Taking into consideration these findings, it can be supposed that keeping the mussels for 3 days, prior to the experiments, in laboratory conditions may result in a greater loss of glycogen from the hepatopancreas and muscle than from the mantle tissues.

The data concerning the glycogen content of the non-gonadal mantle tissues show that in the mussels with a medium length of 3.2 and 4.2 cm the mean glycogen values of 10454 ± 719 and of 7580 ± 780 mg/100 g exceed with 109% ($P < 0.001$) and with 52% ($P < 0.01$) the mean glycogen value of 4999 ± 410 mg% found in the mantle of the mussels with a medium size of 1.7 cm. This increased glycogen deposition in young mussels is probably linked to processes of sexual maturation, the carbohydrate reserves in the mantle — which contribute about 40% in summer to the total body glycogen content [16] — being primarily used in gametogenesis [5].

Our results also suggest that the age corresponding to the size group of 2.2 cm is a critical developmental period, characterized by significantly reduced glycogen amounts in the hepato-pancreas (2012 ± 356 mg%; $P < 0.02$) and the muscle (1283 ± 135 mg%; $P < 0.01$) as compared with those measured in the size group of 1.7 cm (3370 ± 386 mg% for the hepatopancreas and 2653 ± 385 mg% for the muscle). No significant difference is seen in the case of the mantle although a slight tendency for glycogen decrease can be noticed. These changes in the glycogen reserves are in agreement with a sharp drop of about 50% in the circulating glucose level of mussels with a medium body length of 2.0 cm as compared to the values found in the animals with a medium length of 1.5 cm.

CONCLUSIONS

1. In late summer, *Mytilus galloprovincialis* contains large amounts of glycogen distributed unevenly between the three main carbohydrate storing tissues. The highest glycogen content is found in the mantle and the lowest in the muscle.

2. In all the tissues examined, i.e. hepatopancreas, mantle and muscle, the glycogen content shows significant age-related changes which suggest that the ages corresponding to the size groups of 2.2 cm and of 3.2—4.2 cm are critical physiological periods in the development of mussels, reflected in the energy storing capacity of different tissues.

Acknowledgements. The technical assistance of Magdolna Anghelea is acknowledged.

REFERENCES

1. Alemany M., Rosell-Pérez M., 1973, Rev. esp. Fisiol., **29**, 217—222.
2. Alemany M., Rosell-Pérez M., 1974, Rev. esp. Fisiol., **30**, 9—14.
3. Alemany M., Rosell-Pérez M., 1976, Rev. esp. Fisiol., **32**, 81—82.
4. Crăciun C., Molnar B., Madar I., Şildan N., Pora E. A., 1981, Rev. Roum. Biol.—Biol. Anim., **26**, 149—155
5. Gabbott P. A., Bayne L., 1973, J. mar. biol. Ass. U.K., **53**, 269—286.
6. Goddard C. K., Martin A. W., 1966, in: *Physiology of Mollusca* (Eds. K. M. Wilbur, C. M. Yonge), Academic Press, New York-London, **2**, 275—302.
7. Favretto L., 1968, Boll. Soc. Adriat. Sci. nat., **LVI**, 243—261.
8. Madar I., Şildan N., Pora E. A., 1976, St. cerc. biol.— Biol. anim., **28**, 105—108.
9. Madar I., Şildan N., Pora E. A., 1977, Rev. roum. Biol.—Biol. Anim., **22**, 39—43.
10. Madar I., Şildan N., Mihail N., Pora E. A., 1978, Ann. St. Univ. "Al. I. Cuza" Iaşi, (in press).
11. Madar I., Şildan N., Pora E. A., Hernandez L., 1979, Rev. Roum. Biol.—Biol. Anim., **24**, 41—43.
12. Montgomery R. 1957, Arch. Biochem. Biophys., **67**, 378—386.
13. Walne P. R., 1970, Fishery Invest. Series II, **XXVI**, 1—33.
14. Williams C. S., 1969, J. mar. biol. Ass. U.K., **49**, 161—173.
15. Zwaan A. De, Zandee D. I., 1971, Comp. Biochem. Physiol., **43A**, 53—58.
16. Zwaan A. De, Zandee D. I., 1972, Comp. Biochem. Physiol., **43B**, 47—54.

Received June 12, 1980

The Biological Research Centre
Cluj-Napoca, Str. Clinicilor 5—7

HISTOLOGICAL EVIDENCE OF THE ACTION OF SOME ALPHA- AND BETA-CYTOTOXIC COMPOUNDS UPON THE ISLET-LIKE FORMATIONS IN THE INTESTINAL MUCOSA OF *MYTILUS GALLOPROVINCIALIS* (L)

BY

CONSTANTIN CRĂCIUN, BÉLA MOLNAR, IOSIF MADAR, NINA ŞILDAN and EUGEN A. PORA

The actions of alloxan and streptozotocin (both beta-cytotoxic agents) as well as of cobaltous chloride (an alpha-cytotoxic substance) upon the islet-like formations in the intestinal mucosa of *Mytilus galloprovincialis* (L) were studied. It was established that alloxan and streptozotocin destroy the basophil cells (the insulin-like producing ones), while $CoCl_2$ mainly destroys the acidophil cells (probable the glucagon-like material secreting ones) present in the insular formations of this species.

It has recently been demonstrated that insulin-like substances and insulin-like producing cells are present either in the hepatopancreas [15] or in the intestinal mucosa of some gastropodes and bivalve molluscs [2], [4], [6], [9], [11], [15]. On the other hand, so far, there is no sure proof of the existence of a glucagon-like hormone in invertebrates. In spite of this fact, Assan et al. [1] have reported the presence in invertebrates of some glucagon-like immunoreactive substances. Nevertheless, the attempt of Fritsch et al. [5] to put in evidence glucagon-like material producing alpha-cells in the intestinal epithelium of *Mytilus edulis* was unsuccessful.

In this respect, the experiments of Madar et al. [7]—[11] concerning the age-dependent blood glucose dynamics, the "in vivo" effect of insulin upon the blood glucose dynamics, the "in vitro" action of insulin upon the glucose uptake by mantle tissue pieces and the "in vivo" effect of alloxan, streptozotocin and $CoCl_2$ upon the circulating blood glucose levels in *Mytilus galloprovincialis*, are remarkable, strongly suggesting the presence of insulin- and glucagon-like substances in this species as well as the existence of their corresponding cellular structures. These data led us to the present experimental study, in which the selective beta-cytotoxic effect of alloxan and streptozotocin as well as the specific alpha-cytotoxic action of $CoCl_2$ (well known in vertebrates) upon the insular formations in the intestinal mucosa of *Mytilus galloprovincialis* was investigated by light microscopy.

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, N° 2, P. 149—153, BUCAREST, 1981

MATERIALS AND METHODS

The mussels were collected between 14—26 June 1979, from the littoral zone of the Black Sea at Agigea (IRCM-Constanța). Individuals of 5—6 cm length, corresponding to an age of 2—3 years [7], [8], were selected before the experiments, and kept for 5 days in an aquarium in sand filtered, aerated, natural sea water with a total salinity of 15‰, at a temperature of 22°C. The mussels were then distributed in 4 experimental groups of 15 individuals each, as follows : *Group No. 1*, control animals injected with saline solution (100 µl/animal).

Group No. 2, animals injected with a single dose of 12.1 mg alloxan ("Austranal") per 100 g.b.w. solved in saline solution.

Group No. 3, animals injected with a single dose of 20 mg streptozotocin ("Boehringer", Manheim GmbH) per 100 g.b.w. solved in saline citrate buffer (pH = 4.5).

Group No. 4, animals injected with a single dose of 20 mg $CoCl_2$ ("Merck") per 100 g.b.w. solved in saline solution.

The solutions were injected into the hepatopancreas *via* the ligament by means of a microsyringe, having an adjustable needle No. 20.

The animals from groups No. 1, 2 and 3 were put into the fixation liquid 24 hours after receiving the injections and those from the group No. 4, six hours after being injected.

The mussels were fixed in Bouin's solution for 24 hours and then embedded in paraffin and stained, for the demonstration of insular formations, by the Yatsumitsu-Harumichi-Humio technique [17]. It must be mentioned that for histological demonstration and location of the islet-like structures, total and serial sections of the whole body of the mussels were performed. Also, in order to establish certain morpho-physiological correlations, parallelly the same groups of animals were studied from the physiological point of view [10], [11].

RESULTS AND DISCUSSIONS

The histological data, obtained by means of serial sectioning, showed the presence of islet-like structures mainly at the level of the intestinal mucosa. These structures are especially numerous in the intestinal segment from the dorsal part of the animal, i.e. in the vicinity of the heart and at the emergence of the intestine from the hepatopancreas. Isolated insular formations vere also found at the level of hepatopancreas. In this respect, Fritsch et al. [5], [6] reported that in *Mytilus edulis*, the insulin-like material producing cells are situated in the mucosa of the intestinal segment which passes through the hepatopancreas.

The preponderant location of the islet-like structures of *Mytilus galloprovincialis* in the intestinal segment crossing the pericardial cavity is probably, as suggested before [10], [11], connected with a direct hormonal regulation of glucose levels in the haemolymph of this species.

Group No. 1 : Control mussels. In the control mussels, the islet-like structures in the intestinal mucosa are present in a great number, consist-

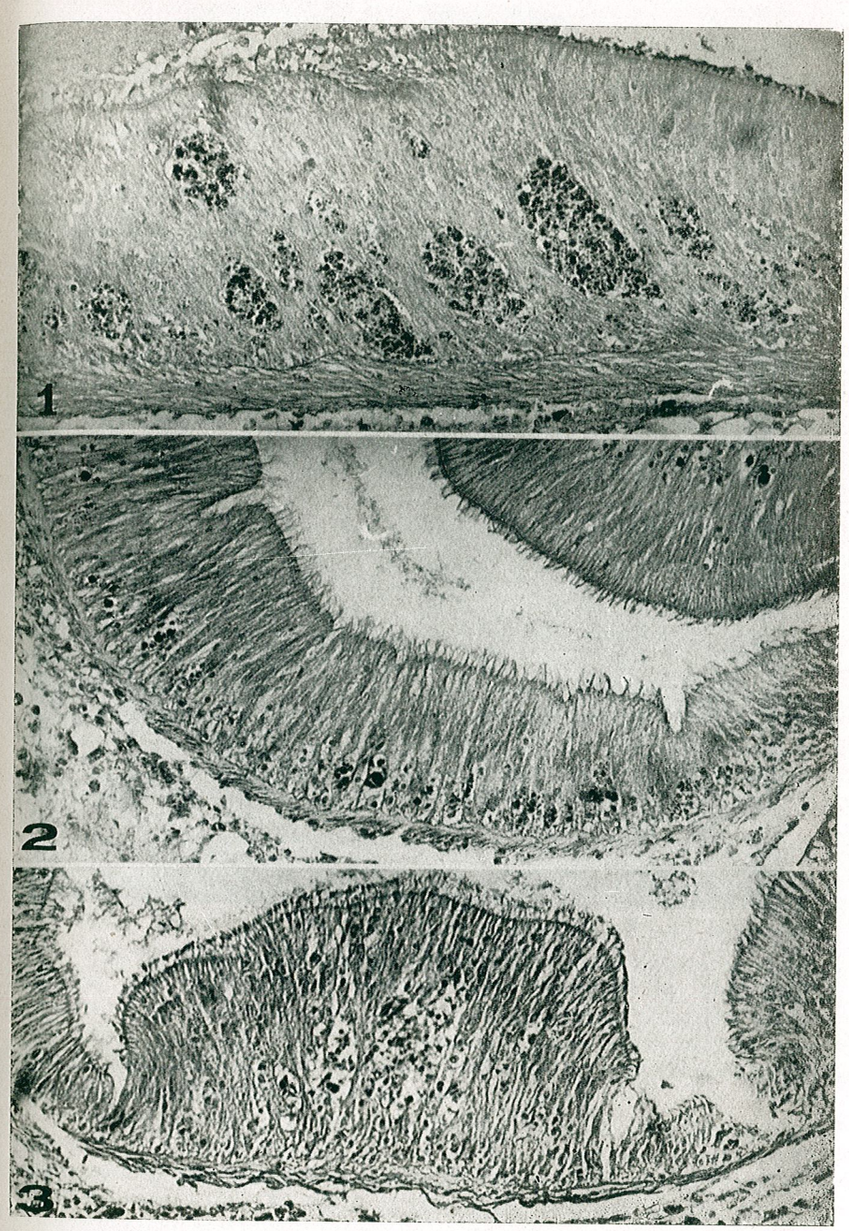


Fig. 1. — Control group. Numerous insular formations and cellular aggregations in intestinal mucosa of *Mytilus galloprovincialis* (L). Ob. × 25.
Figs. 2. — and 3. — Alloxan injected group. Fig. 2. Strongly affected insular formations are visible. Ob. × 25. Fig. 3. Pronounced cellular lysis in the insular formations. Ob. × 25.

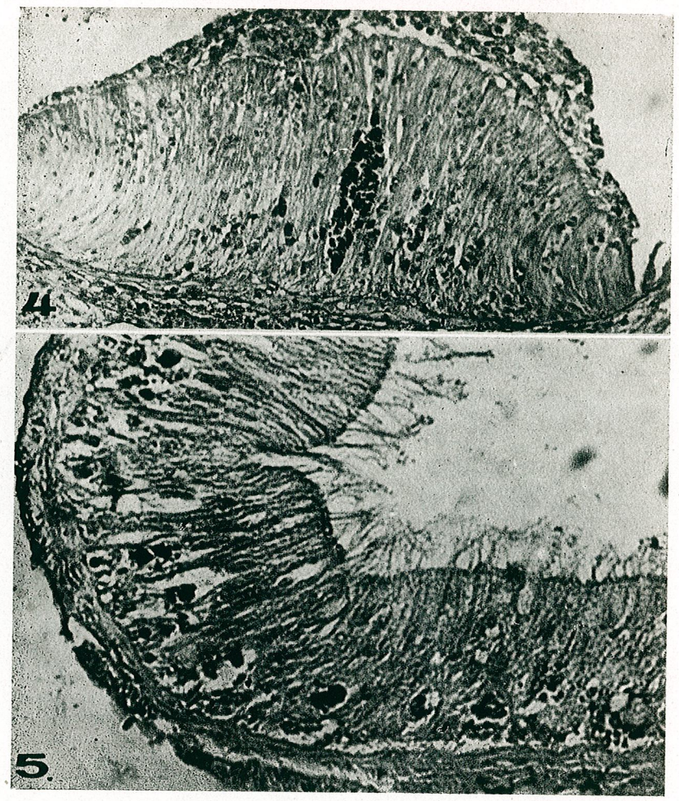


Fig. 4. — Streptozotocin injected animals. Partial damaged insular formations and cellular aggregations. Ob.× 25.
Fig. 5. — Cl_2Co injected mussels. The general affectation of insular formations. A reduced number of undamaged cellular aggregates represent beta cells. Ob.× 50.

ing of either cellular congregations, forming islets of large sizes, or small cellular aggregates (2—3 insulin-like secreting cells) scattered in the intestinal epithelium(Fig. 1). When situated in the low intestinal epithelium, the islets are spherical or sometimes may have an irregular outline. The islets located in the tall intestinal epithelium have an oval shape.

The distribution of insular formations along the intestinal mucosa is uneven. In certain regions they are in great number while in others they are scarce. Regardless of their distribution the insular cells contain a great number of secretory granules. The turgescent appearance of the cells as well as the granular content of their cytoplasma, revealed with the aid of the specific staining method used, indicate an intense activity of formation of the secretory material.

In the period when the experiments were carried out (June), the greatest majority of insular formations exhibited tinctorial affinities for Victoria blue and chromium hematoxylin, that is basophil affinities characteristic of the beta, i.e. insulin secreting cells. Rarely, groups of 2—3 phloxynophil cells were observed, indicating the presence, in a reduced number, of the alpha, glucagon-like secreting cells.

Group No. 2: *Alloxan treated mussels.* The physiological studies carried out on this group of mussels [10], [11] showed a 70% increase of the blood sugar level, as compared to the control group. Histologically, it was demonstrated in mammals that alloxan produces a selective necrosis in the beta cells and that in this case the pancreatic islets contain only alpha cells [13], [14]. The selective destruction of beta cells is always accompanied by a definite hyperglycemia.

Our histological findings demonstrate that after alloxan treatment the insular formations in mussels are strongly affected (Figs. 2 and Fig.3). This is expressed by the reduction of the dimensions of islet-like formations (Fig. 2) and by large areas of cellular lysis which, in some insular structures, may affect almost all the cells (Fig. 3).

Many islets and cellular aggregates depict marks of characteristic disintegration : the cells become angular with irregular shape, the intense basophil cytoplasma is reduced and disposed in a narrow band around the nuclei. Many cells are completely degranulated and vacuolized.

Taking into consideration the selective beta-cytotoxic effect of the alloxan and the strong cellular lysis observed in the insular formations of *Mytilus galloprovincialis*, we appreciate that the disintegrated and altered cells are beta insulin secreting ones. Also, it may be assumed that at least partially the unaffected cells in the islets represent alpha, i.e. glucagon-like material secreting ones.

Group No. 3 : *Streptozotocin treated mussels.* The physiological data indicated a marked hyperglycemia in this group of mussels (+122%) as compared to the control animals. Streptozotocin as alloxan is a beta-cytotoxic agent [16]. In spite of the sharp increase of the blood glucose, the histological sections show that the insular formations and the cellular aggregates, after streptozotocin treatment, do not undergo such pronounced alterations as in the alloxan treated group.

Thus, in the intestinal mucosa, one can still find sparce islets and cellular aggregates apparently unaffected (Fig. 4). However, in the great majority of the islets, partial cellular lysis of the insulin-like secreting

cells accompanies the volume reduction of the insular formations. Also a great decrease in the total number of the islets is noted.

It may be assumed that, in streptozotocinized mussels, as in the case of alloxanized ones, the damaged cells are the beta, i.e the insulin secreting cells.

Group No. 4: $CoCl_2$ treated mussels. The specific alpha cytotoxic effect of cobaltous chloride is well known in mammals [3], [12], [16]. The transitory hyperglycemia which follows the $CoCl_2$ administration is ascribed to glucagon release from the alpha cells before undergoing the characteristic degenerescence due to the selective toxic action of this substance [12], [13].

In mussels, Madar et al. [10], [11] found a 144% increase of blood glucose level, as compared to the control group, 6 hours after injecting $CoCl_2$. This finding gave support to the supposition that the rise of blood glucose content in this case could be ascribed, by analogy, to the liberation of a glucagon-like factor, promoting the mobilization of glucose from hepatopancreas and other glycogen storing tissues.

In this group of $CoCl_2$ treated mussels we tried to check the presence of alpha cells in the islet-like structures.

Generally, the histologic slides show a decrease in the number and a reduction in the volume of the islets accompanied by a pronounced cellular lysis (Fig. 5). The light microscopically observed cellular damages induced by $CoCl_2$ are particularly severe, the noxious effect of this substance extending to a certain degree to the non-alpha endocrine elements indicates its high toxicity. However, it may be considered that the unaffected cell groups in the islet formations of *Mytilus galloprovincialis* are mostly beta, insulin secreting cells.

The alteration induced by alloxan and streptozotocin in the beta cells of the islet formations located in the intestinal mucosa of *Mytilus galloprovincialis* parallelled by a definite hyperglycemia certify the fact that the beta-cells in the mussels are insulin secreting cells. Also, our histological data, obtained in control as well as in $CoCl_2$ treated mussels, indicate that beside the acidophil beta cells, glucagon-like material secreting alpha cells are also present in the insular formations of *Mytilus galloprovincialis*.

From this point of view, our results are in good agreement with the data of Madar et al. [10], [11] and support the hypothesis that, besides the cellular metabolic regulation of the carbohydrate metabolism in *Mytilus galloprovincialis*, there exists a hormonal regulation through the secretion of insulin- and glucagon-like substances.

REFERENCES

1. Assan R., Tchobroutsky G., Rosselin G., 1969, Path. Biol., **17**, 747—755.
2. Boquist L., Falkmer S., Mehrotra B. K., 1971, Gen. comp. Endocrinol., **17**, 236—239.
3. Eaton R. P., 1973, Amer. J. Physiol., **225**, 67—72.
4. Falkmer S., Emdin S., Savu N., Lundgreen G., Marques M., Östberg Y., Steiner D. F., Thomas N. V., 1973, Amer. Zool., **13**, 625—638.
5. Fritsch H. A. R., Van Noorden S., Pearse A. G. E., 1976, Cell Tissue Res., **165**, 365—369.

6. Fritsch H. A. R., Sprag R., 1977, Cell Tissue Res., **177**, 401—413.
7. Madar J., Şildan N., Pora E. A., 1976, Stud. cerc.-biol., Ser. Biol. Anim., **28**, 105—108.
8. Madar J., Şildan N., Pora E. A., 1977, Rev. Roum. Biol.—Biol. Anim., **22**, 39—43.
9. Madar J., Şildan N., Mihail N., Pora E. A., 1978, Ann. St. Univ. "Al. I. Cuza" Iaşi. (in press).
10. Madar J., Şildan N., Pora E. A., 1979, Rev. Roum. Biol.—Biol. Anim., **24**, 41—43.
11. Madar J., Şildan N., Pora E. A., Crăciun C., Molnar B., 1980 (in press).
12. Martin G. G., Bruno W. V., Sydney S. L., 1952, Metabolism, **I**, *6*, 544—548.
13. Martin G. G., Bruno W. V., Sydney S. L., 1954, J. Clin. Endocrinol. Metab. **XIV**, *2*, 184—192.
14. Martinez De R. N., Garcia C. M., Salas M., Candela J. L. R., 1973, Gen. Comp. Endocrinol., **20**, 305—411.
15. Plisetskaya E., Kazakov V. K., Soltiskaya L., Leibson L. G., 1978, Gen. comp. Endocrinol., **35**, 135—145.
16. Violettes B., Sutherland E. R. D., Matas J. A., Payne W., Najarian S. J., 1979, Metabolism, **28**, 489—494.
17. Yatsumitsu K., Harumichi S., Humio M., 1977, Acta Histochem. cytochem., **10**, 10—13.

Received July 15, 1980

The "Babeş-Bolyai" University,
Department of Biology and The Biological
Research Centre, Cluj-Napoca, Str. Clinicilor 5—7

EFFETS D'UN RAYONNEMENT MICROONDE (2450 MHz) SUR CERTAINES FONCTIONS ENDOCRINES DE LA SOURIS*

PAR

P. DESCHAUX, C. JOUVE DE GUIBERT, R. SANTINI et J. P. PELLISSIER

The biologic effects of an electromagnetic field with a frequency of 2450 MHz were studied in mice in a multimode chamber.
The results of investigations have shown that microwave irradiation of 10 mW/cm² for 5 days, 1 hr/day or 2 hr/day, or 3 hr/day or 4 hr/day causes an increase in luteotrophic hormone, adrenocorticotrophic hormone, testosterone and corticosterone plasma levels with no structural alterations. These perturbations were transitory.

INTRODUCTION

Le nombre important des applications industrielles, domestiques, thérapeutiques des microondes expose, actuellement, les populations des pays industrialisés à des taux importants de radiation durant de longues périodes. La règlementation française a défini des normes d'exposition. Ces valeurs limites d'exposition n'ont qu'une valeur indicative et ne peuvent être considérées comme une frontière entre les ambiances dangereuses et celles qui ne le sont pas, principalement en raison de l'importance des variations de la sensibilité individuelle. Les valeurs recommandées sont fonction de 2 paramètres : densité de puissance et durée totale d'exposition.

Le travail que nous rapportons se situe dans le cadre de l'étude de l'effet nocif éventuel des microondes. Nous avons utilisé une densité de puissance de 10 mW/cm², valeur autorisée par les normes françaises, avec des durées d'irradiation de 1, 2, 3 ou 4 heures, quotidiennement et pendant 5 jours.

Nous avons travaillé sur des souris mâles agées de 1 ou 2 mois, en étudiant l'effet des microondes sur 2 systèmes endocriniens : l'axe hypophysotesticulaire et l'axe hypophyso-corticosurrénalien.

MATÉRIELS ET MÉTHODES

Animaux : Nous utilisons des souris Swiss mâles agées de 30 à 60 jours.

Conditions d'irradiation : Les animaux sont soumis à une irradiation continue microonde (2450 MHz) sous une puissance de 10 mW/cm² dans

* Ce travail a bénéficié de l'aide du Centre National de la Recherche Scientifique (Contract n° 3086).

une cavité multimode (dimensions : hauteur, 32,5 cm ; largeur 32 cm ; longueur, 54 cm) pendant un temps quotidien de 1, 2, 3 ou 4 heures durant 5 jours consécutifs.

La puissance P appliquée est calculée d'après la formule :

$$P(\text{watts}) = N \times S \times 10^{-2}$$

(N étant le nombre d'animaux placés dans la cavité, S la surface moyenne du corps d'une souris).

Durant les expériences, les animaux reçoivent nourriture et boisson ad libitum. Les animaux témoins sont traités de la même façon sans irradiation. La température intérieure de la cavité est maintenue constante à 23—25°C. Les animaux sont disposés par lot de 5 individus dans des cages en plastique mises dans la cavité multimode.

Les animaux sont sacrifiés par décapitation soit immédiatement, soit 24 heures, soit 5 jours après la dernière irradiation. Le sang est prélevé et le plasma séparé et stoké à — 20°C pour les dosages d'hormones. Les testicules sont prélevés pour l'étude in vitro de la sécrétion de testostérone, les glandes corticossurrénales pour l'étude de la sécrétion de corticostérone. Nous avons étudié le taux de sorbitol déhydrogénase (SDH) enzyme caractéristique de l'activité testiculaire [8]. Enfin nous avons également réalisé une étude histologique des 2 glandes.

Dosages des hormones :

a — corticostérone : le taux de corticostérone est déterminé selon la technique de radiocompétition protéique de Jolivet [4] et de Murphy [9]. Nous avons utilisé le transcortine de rat comme « binding protein » ; les stéroïdes entrant en compétition avec la corticostérone sont éliminés par extraction du plasma avec du tétrachlorure de carbone, suivie d'une chromatographie sur célite. L'hormone libre est séparée de l'hormone liée à l'aide d'une solution de charbon dextran.

b — Testostérone : nous avons utilisé la méthode radioimmunologique décrite par Nieschlag et Loriaux [10] utilisant l'anticorps fourni par ces auteurs. La testostérone est séparée de la déshydrotestostérone par chromatographie sur célite (Mahoudeau et Bricaire [6]).

Le coefficient de variation intra-assay est inférieur à 10% ; une valeur de 10 µg est significative ; le taux de récupération de testostérone après l'étape chromatographique est d'environ 7%.

c — ACTH : nous utilisons un antisérum de lapin obtenu à partir d'injection d'ACTH porcine (ACTH retard de porc Choay [1]). L'anticorps est produit principalement contre le fragment biologique 1,24 ACTH. L'ACTH est adsorbée avec 50 mg de talc. La radioactivité est déterminée dans le précipité après centrifugation. La sensibilité de notre dosage est de 10 µg/ml : le coefficient intra-assay est de ≤ 8% et le coefficient interassay est de ≤ 11%.

d — LH : le taux plasmatique de LH est déterminé par radioimmunoassay selon la technique décrite dans le Kit fourni par le « Rat Pituitary Distribution Programm NIAMD ». La sensibilité du dosage est de 20 µg, le coefficient intraassay de 7%, le coefficient interrassay de 12%.

Étude du taux de sécrétion des hormones (testostérone-corticostérone) in vitro

Les glandes corticosurrénales et les testicules sont prélevés, pesés et immédiatement mis à incuber dans 5 ml de milieu de culture 199. Après une incubation de H à 37°C, sous courant gazeux (CO_2 5% ; O_2 95%) et agitation, 1 ml de milieu est prélevé et les taux d'hormones (testostérone, corticostérone) dont déterminés selon les techniques décrites précédemment.

Étude du taux de sorbitol déshydrogénase (SDH) testiculaire

a — *Préparation du tissu* : les testicules prélevés sur des animaux tués par décapitation sont pesés et rapidement introduits dans une solution physiologique froide (4°C). On enlève la tunique albuginée et on broie à l'ultraturax dans un tampon phosphate sodium pH 7,0 à raison de 2 ml/g de poids. Après ultracentrifugation à 105 000 g (30 mn) le surnageant est récupéré et utilisé pour la détermination de l'activité spécifique de la SDH.

b — *dosage* : la SDH assure la transformation réversible du sorbitol en fructose en présence de NADH, dont on suit l'extinction au photocolorimètre, en ultra violet, suivant la réaction :

$$\text{Sorbitol} + \text{NAD} \xrightarrow{\text{SDH}} \text{Fructose} + \text{NADH}$$

Nous utilisons la technique de Gerlachet Schurmeyer [3] qui étudient soit l'extinction de NADH, soit l'effet de la réaction dans le sens fructosesorbitol.

Dans des tubes à essai nous ajoutons :

— tampon trithanolamine pH 7,4	0,2 M	2 ml
— solution à doser		1 ml
— NADH	12 mM	0,05 ml

Après incubation du mélange à 25°C pendant 45 mn, nous ajoutons 0,2 ml de fructose 1,65 M.

Étude histologique

Les organes (testicules-corticossurénales) sont fixés dans une solution de Bouin. Après fixation pendant 3 jours, les organes sont lavés à l'eau distillée puis à l'alcool à 70° jusqu'à élimination complète de l'acide picrique. Les organes sont ensuite deshydratés à l'alcool et inclus dans la paraffine et coupés sagittalement au microtome à une épaisseur de 5 µ.

Les coupes obtenues sont paraffinées au toluène (3 bains de 10 nm), lavées à l'alcool absolu et déshydratées à l'alcool à 95° et 70°.

Les différentes colorations effectuées sont :

— pour la coloration nucléaire, l'hématoxyline de Groat : bain dans le colorant pendant 1 mn environ puis rinçage à l'eau courante,

— pour la coloration cytoplasmique, éosine à 1% : coloration 1 mn maximum puis lavage à l'eau courante.

Le montage se fait dans le baume de Canada.

L'observation se fait au microscope photonique.

RÉSULTATS

I — *Effet du rayonnement microonde sur le taux d'hormones circulantes*

1 — *Testostérone.* Nos résultats sont rapportés sur le tableau 1. Nous observons une augmentation du taux de testostérone plasmatique après l'irradiation microonde, cette modification est décelable lorsque les animaux sont sacrifiés imédiatement et 24 heures après la dernière irradiation. L'âge des animaux n'intervient pas.

2 — *LH.* Les résultats obtenus pour le dosage de la LH plasmatique sont rapportés sur le tableau I. Les variations obtenues sont observées dans les mêmes cas que pour la testostérone.

3 — *Corticostérone.* Nous observons (tableau 2) une augmentation du taux de corticostérone plasmatique jusqu'à 24 heures après la dernière irradiation quelle que soit la durée de celle-ci. L'âge de l'animal n'a pas d'influence.

4 — *ACTH.* Les taux plasmatiques d'ACTH sont rapportés sur le tableau II. Les variations obtenues sont superposables à celles observées pour la corticostérone.

II — *Etude des sécrétions d'hormones in vitro.* Aucune différence entre les organes (testicules et corticosurrénales) des animaux irradiés ou non a été observée.

III — *Etude histologique.*

Aucune modification histologique cellulaire n'a été relevée dans les organes (testicules, corticosurrénales) après irradiation microonde. Nous n'avons pas observé de perturbations au niveau de la spermatogénèse.

IV — *Etude du taux de sorbitol déshydrogénase des testicules.*

Nous avons rapporté sur le tableau 3 le taux de SDH testiculaire après irradiation. Aucune variation n'apparaît.

DISCUSSION

Afin de définir les conditions d'utilisation des microondes, de nombreux travaux sont actuellement en cours qui visent à préciser les effets biologiques de ces rayonnements. Selon les intensités et les fréquences auxquelles ces ondes sont utilisées, des effets sont signalés soit dangereux (brûlures, cataractes, inversion de formules sanguines, perturbation du transit digestif) soit utiles (traitement anticancéreux, amélioration des défenses immunitaires, détermination du taux de médiateurs chimiques [5] [12] [11]).

Dans les pays occidentaux les valeurs seuils sont : la minimum 10 mW/cm² où, au-dessous de cette valeur aucune restriction n'intervient, la maximum à 100 mW/cm² où au dessus de cette valeur toute exposition est interdite. Pour les autres pays, les formules et les valeurs sont très différentes. Ce problème de protection est donc délicat à traiter [2].

Tableau 1

Effet chez la souris d'une irradiation microonde sur les taux plasmatiques de testostérone et de LH.

Durée quotidienne de l'irradiation (en heures)	Nombre total de jours d'irradiation	Temps séparant la dernière irradiation du moment du sacrifice	Âge des animaux (jours)	Taux de testostérone plasmatique (ng/100 ml)			Taux de LH plasmatique (ng/ml)		
				Témoin		Irradié	Témoin		Irradié
1	1	0	30	87,2± 2,3	↗	106 ± 2,0	94 ± 0,6	↗	108 ± 0,8
			60	313 ±18,3	↗	341 ±14,0	82 ± 4,4	↗	112 ± 1,1
		24 heures	30	55 ± 2,2	NS	48,7± 7,1	92,8± 6,1	NS	100 ± 4
			60	297,8±12,2	NS	281 ±29	83,4± 0,8	NS	82 ± 2
		5 jours	30	64,2± 4,3	NS	65 ± 3,1	101,4±10,2	NS	96,6±7,3
			60	316 ±18,0	NS	308,8± 5,2	79 ± 1	NS	87 ±22
	2	0	30	76,4± 4,2	↗	100,6± 5,6	91,9± 1	↗	126 ± 2,2
			60	318,4± 8,4	↗	410 ±20,7	92,4± 0,8	↗	101 ± 1
		24 heures	30	66,4± 3,0	↗	76 ± 2,4	100 ± 9,3	↗	118 ± 2
			60	268,2±11,1	↗	294 ± 6,9	79 ± 4,5	↗	94 ± 0,8
		5 jours	30	63,8± 5,0	NS	61,6± 0,9	88,2± 0,9	NS	88 ± 2
			60	243,4±11,0	NS	262 ±10,7	86 ± 2,4	NS	86 ± 2,4
	3	0	30	90,8± 3,9	↗	105 ± 4,5	96 ± 2,4	↗	124 ± 2,4
			60	314,8± 1,3	↗	429 ±85,0	96 ± 5,3	↗	120 ± 3,1
		24 heures	30	88,4± 4,1	↗	121,4± 4,6	80 ± 2,7	↗	120 ± 1,1
			60	312 ±22,8	↗	344 ± 5,0	87 ± 4,3	↗	120 ± 4,4
		5 jours	30	51,4± 3,1	NS	55 ± 5,7	106 ± 5,0	NS	112 ± 4,8
			60	344± 8,7	NS	351 ±11,2	79 ± 3,3	NS	80 ± 2
2	4	0	30	74,2± 2,4	↗	152 ±10,3	91 ± 9	↗	104 ±2,4
			60	376 ± 2,9	↗	475 ±30,9	98 ± 8	↗	128 ± 2
		24 heures	30	88,8± 3,0	↗	162 ± 4,8	105 ± 2,2	↗	122 ± 5,8
			60	392 ±15,9	↗	423 ±3,7	99 ± 3,3	↗	120 ± 7,0
		5 jours	30	93,4± 5,1	NS	107,2± 1,7	96 ± 9,79	NS	101 ±2,69
			60	282 ±4,6	NS	315,4± 9,3	72 ± 2	NS	71 ± 3,3

Tableau 2

Effet chez la souris d' une irradiation microonde sur les taux plastiques de corticostérone et de ACTH

Durée quotidienne de l'irradiation (en heure)	Nombre total de jours d'irradiation	Temps séparant la dernière irradiation du moment du sacrifice	Âge des animaux (jours)	Taux de corticostérone plasmatique (µg/100 ml)			Taux d'ACTH plasmatique (pg/ml)		
				Témoin		Irradié	Témoin		Irradié
1	1	0	30	14,8± 0,5	↗	18,6± 2,1	108 ± 3,7	↗	124 ± 2,4
			60	15,2± 0,8	↗	25,4± 2,0	76 ± 6	↗	124 ± 4
		24 heures	30	14,6± 0,8	↗	18,6± 0,4	102 ± 2	↗	122 ± 2
			60	12 ± 0,4	↗	14,6± 0,9	85 ± 5,3	↗	107 ± 8
		5 jours	30	14,6± 1,4	NS	14,4± 1,1	118 ± 4,8	NS	119 ± 5,5
			60	14,6± 0,7	NS	13,2± 0,3	97 ± 2,5	NS	104 ± 6
2	2	0	30	14,2± 0,5	↗	18,4± 0,4	101 ± 4	↗	117 ± 2
			60	12,2± 0,8	↗	16,4± 1,3	80 ± 3,5	↗	122 ± 3,3
		24 heures	30	14,8± 0,3	↗	18 ± 1,6	117 ± 4,8	↗	124 ± 4
			60	15,6± 0,3	↗	17,4± 1,4	58 ± 1,6	↗	90 ± 3,6
		5 jours	30	12 ± 0,3	NS	12,8± 1,2	83 ± 7	NS	84 ±14,6
			60	12,5± 0,6	NS	12 ± 0,3	92 ± 8	NS	105 ± 6,3
3	3	0	30	13,2± 0,8	↗	16,8± 0,7	120 ± 8,9	↗	134 ± 4
			60	14,6± 1,0	↗	18,6± 0,3	90 ± 4,1	↗	122 ± 2
		24 heures	30	13 ± 0,6	↗	18,6± 1,6	118 ± 5,8	↗	132 ± 3,7
			60	14 ± 0,7	↗	18 ± 1,5	81 ± 4,8	↗	122 ± 2
		5 jours	30	13,2± 0,7	NS	12,4± 1,8	110 ± 3,4	NS	102 ±11,1
			60	13 ± 1,6	NS	12 ± 0,5	96 ± 8,1	NS	98 ± 9,6
4	4	0	30	10,8± 0,1	↗	20 ± 1	81 ± 3,3	↗	108 ± 7,3
			60	12,4± 0,3	↗	19,2± 0,5	66 ± 9,1	↗	112 ± 9,6
		24 heures	30	18,2± 0,7	↗	21 ± 0,3	38 ± 5,8	↗	56 ± 3,6
			60	17,6± 1,4	↗	20 ± 2,0	45 ± 6,3	↗	73 ± 9,5
		5 jours	30	12 ± 0,3	NS	12,2± 0,3	76 ± 2,4	NS	78 ± 9,5
			60	17,2± 0,2	NS	17,6± 1,4	74 ± 7,4	NS	78 ± 5,8

Tableau 3

Effet chez la souris d'une irradiation microonde sur le taux testiculaire de sorbitol déhydrogénase

Temps d'irradiation par jour	nombre de jours d'irradiation	date du sacrifice	âge	témoin		irradié
4	1J	0	30J	1,3±0,05	NS	1,2±0,01
			60J	3,5±0,04	NS	3,6±0,05
		24H	30J	1,8±0,02	NS	1,7±0,07
			60J	4,0±0,03	NS	3,9±0,06
		5J	30J	1,5±0,09	NS	1,5±0,06
			60J	3,2±0,05	NS	3,3±0,07
	2J	0	30J	1,5±0,02	NS	1,5±0,07
			60J	3,5±0,01	NS	3,7±0,02
		24H	30J	1,2±0,04	NS	1,3±0,05
			60J	3,2±0,03	NS	3,1±0,05
		5J	30J	1,8±0,06	NS	1,7±0,04
			60J	4,1±0,05	NS	3,8±0,05
	3J	0	30J	1,6±0,05	NS	1,6±0,06
			60J	3,8±0,02	NS	3,8±0,08
		24H	30J	1,6±0,07	NS	1,4±0,06
			60J	3,9±0,01	NS	3,8±0,07
		5J	30J	1,6±0,01	NS	1,5±0,06
			60J	3,2±0,02	NS	3,5±0,06
	4J	0	30J	1,3±0,05	NS	1,3±0,04
			60J	3,5±0,07	NS	3,2±0,03
		24H	30J	1,4±0,05	NS	1,4±0,02
			60J	3,8±0,02	NS	3,4±0,05
		5J	30J	1,4±0,03	NS	1,6±0,05
			60J	3,4±0,04	NS	3,4±0,05

Dans le cadre d'une étude sur la radio-protection nous nous sommes intéressés aux effets biologiques d'un rayonnement microondes à deux puissances faible (10 mW/cm²) de courte durée, alors que la majeure partie des travaux rapportés sont réalisés à des valeurs de puissance hyper-

thermiques et de longue durée. De plus, en faisant varier le temps séparant la dernière irradiation du moment du sacrifice nous avons recherché l'existence d'un effet immédiat du rayonnement microonde.

Pour cette étude nous avons utilisé des souris mâles Swiss, âgées de 30 jours (âge de la puberté chez cette espèce) et de 60 jours ; nous avons étudié l'effet d'un rayonnement microonde, continu en cavité multimode. Cette irradiation se fait à une puissance de 10 mW/cm² de surface animale et pendant 1, 2, 3 ou 4 heures pendant 5 jours. Les effets biologiques ont été étudiés au niveau des axes hypophysotesticulaire et hypophysocortico surrénalien. Dans le premier cas, nous avons étudié les taux plasmatiques d'hormone lutéotrope (LH) et de testostérone, ceci soit immédiatement, soit 24 heures, soit 5 jours après la dernière irradiation ; de même, il a été étudié les taux d'hormone adrénocorticotropique et de corticostérone.

Nous avons montré qu'il y avait une augmentation des taux circulant de testostérone et de LH et ce résultat est obtenu lorsque le sacrifice des animaux est pratiqué immédiatement après la dernière irradiation ou après 24 heures ; cet effet se manifeste à partir d'une irradiation de 2 heures. Aucune modification n'est observée 5 jours après l'irradiation.

L'irradiation crée donc un déséquilibre de la sécrétion de l'axe hypophyso-testiculaire sans possibilité de régulation par un effet feed back. Aucune modification structurale n'est observée au niveau des images histologiques de testicules démontrant l'absence de perturbations de la spermatogénèse. La descendance des animaux irradiés est normale sur les plans numérique et morphologique. De plus, les testicules des animaux irradiés, isolés et mis en culture ne montrent pas une sécrétion supérieure à ceux des animaux témoins non irradiés ; enfin le taux de sorbitol deshydrogénase, enzyme caractéristique du métabolisme testiculaire, n'est pas modifié.

Le taux de corticostérone est également augmenté dans les mêmes conditions que pour la testostérone et ceci sans atteinte de la structure cellulaire glandulaire et sans perturbation de la sécrétion in vitro.

La variation de l'hormone adrénocorticotrope est parrallèle et de même sens.

Cette perturbation de l'axe hypophyso-surrénalien démontre que les microondes provoquent un stress, les animaux «témoins» maintenus dans une cavité multimode identique n'ont pas de modification de leurs sécrétions. L'animal subit donc un stress pouvant se situer à un niveau thermique punctiforme, avec présence de points de focalisation ; cette dernière hypothèse reste à démontrer. Par contre l'effet thermique s'il est en cause, n'est pas un effet thermique général, car la température centrale des animaux irradiés n'a jamais été perturbée.

Nous pensons que cette perturbation observée au niveau de l'axe hypophyso-corticossurrénalien entraîne une perturbation plus générale du système endocrinien ce qui peut expliquer les perturbations que nous trouvons au niveau de l'axe hypophysotesticulaire et observées par d'autres auteurs au niveau de la glande thyroïde et de l'hypophyse [7].

Nos résultats font également apparaître un effet transitoire des microondes émises à faible puissance. Ceci est en accord avec les travaux de Michaelson et coll. [7] qui retrouvent cet effet transitoire au niveau

de la sécrétion de l'hormone de croissance ; cet effet est attribué à des perturbations thermiques influençant le système hypothalamo-hypophysaire.

L'observation de cet effet transitoire est également en accord avec les rapport des médecins du travail signalant des troubles réversibles chez des techniciens exposés à de faibles doses de rayonnements (10 mW/cm²), effets réversibles lorsque l'individu est sorti du rayonnement.

Ces effets ont amené les différents auteurs à parler de syndrome de microondes, qui serait dû plus à une sensibilisation de l'organisme aux nuisances de l'environement, qu'à une conséquence directe de l'exposition aux microondes.

BIBLIOGRAPHIE

1. Cros G., Vague P., Oliver C. et Depieds R., C.R. Soc. Biol., 1970, **164**, 1289–1292.
2. Dodge C. H. et Glaser Z. H., J. of Microw. Pow., 1977, **12**, 319–334.
3. Gerlach W. et Shurmeyer E., Z. Ges. Exp. Med., 1960, **132**, 413–439.
4. Jolivet A., Ann. Endocrinol. (Paris), 1979, **38**, 223–229.
5. Leroy Blank C., Sasa S., Isernhagen R., Meyerson L. R., Wassil D., Wong P. Modak A. T. et Stavinoha W. B., J. Neuroch., 1979, **33**, 213–219.
6. Mahoudeau J. A. et Bricaire H. Ann. Biol. Clin. (Paris), 1972, **30**, 559–566.
7. Michaelson S. M., Guillet R., Lotz W. G., Shin-Tsul et Magin R. L., Rockville (Maryland) 1977.
8. Mills N. C. et Means A. R., Endocrinology, 1972, **91**, 147–156.
9. Murphy B. E. P., J. Clin. Endocrinol. Metab., 1967, **27**, 973–990.
10. Nieschlag E. et Loriaux D. L., Z. Klin. Chem. Biochem., 1972, **10**, 164–168.
11. Robert D., Deschaux P., Pellissier J. P., Santini R. et Fontanges R., *Etude préliminaire de l'effet d'un rayonnement microonde 2450 MHz sur les fonctionnements immunitaires de la souris.*
12. Santini R., Deschaux P., Jacomino J. M. et Pellissier J. P., J. of Microw. Pow., 1979, **14**, 287–289.

Reçu le 15 janvier 1981

Laboratoire de physiologie cellulaire
et physique expérimentale
Université Claude Bernard
69622 Villeurbanne, Cedex
France

FORMS AND CONDITIONS OF ACHIEVEMENT OF THE MEMBRANE RESPONSE AT VARIOUS VALUES OF THE EXTERNAL $K^+ : Ca^{2+}$ RATIO

BY

ȘT. AGRIGOROAEI, I. NEACȘU, V. CRĂCIUN, GABRIELA AGRIGOROAEI

The phospholipidic micellae of both types (laminar and globular) are differentiated in what concerns the degree of sensitivity and their number function of the degree of sensitivity towards the specific destructuring ion and depending on the direction (increase or decrease) and the way of the $K^+ : Ca^{2+}$ ratio variation (by modifying the concentration of either K^+ or Ca^{2+}). The depolarizations, hyperpolarizations and repolarizations of the membrane with respect to this ratio are rapid and sometimes ample, when resulting from the increase of the concentration of the destructuring ion, and slow and of a small amplitude, when triggered by the reduction in concentration of the specific structuring ion.

It has previously been shown [2], [3] that the passive responses of the membrane to the ratios and the ionic concentrations in the medium can be explained on the basis of the independent capacity of manifestation of the membrane layers, due to the differentiation of the supramolecular phospholipidic structures in each layer into structures which are sensitive to either the increase or the reduction of the ionic ratios, and under the differentiating of the elements of one and the same type of structure in what concerns the degree of sensitivity towards the specific destructuring agent, and their number corresponding to every degree of sensitivity.

In the passive depolarizations and hyperpolarizations of the membrane, resulting from the variations of the external $K^+ : Ca^{2+}$ ratio, the external layer of the membrane (both with its cationitic closed laminar structures and its anionitic open globular ones) is implied.

The phase transition of the closed micellar structures leads to their changing into open micellar structures and *vice-versa* [2], [10].

The laminar micellae constitute the "area" of the structures which are sensitive to the increase of the $K^+ : Ca^{2+}$ ratio (and, consequently, of depolarizations), and the globular micellae that of the structures sensitive to the reduction of this ratio (and thus of hyperpolarizations).

The depolarization of the membrane when either the concentration of extracellular K^+ is increased [1], [7], [14], or Ca^{2+} is absent [5], [6], [15], and its hyperpolarization when either extracellular K^+ is absent [1], [14] or external Ca^{2+} is increased in concentration, [8], [9], [11], have already been described by many authors. Still, they have failed to provide a satisfactory explanation both on the intimate mechanisms of these phenomena and the conditions in which they take place as well as

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, Nº 2, P. 165—169, BUCAREST, 1981

the conditions leading to important and significant differences in the subsequent evolution of these phenomena.

The re-investigation of these phenomena and the interpretation of the results in the "2-M.S.I." view [2], [3], [4], [12], [13] led to a more unitary and accurate interpretation, followed by general remarks.

The results of experiments have clearly shown that the depolarization obtained by increasing K^+ concentration sets up rapidly and when the concentration is highly increased it can be ample. But, if the depolarized membrane is introduced into a normal physiological solution, the repolarization takes, on the whole, more time than the depolarization.

On the contrary, the depolarization taking place in a low Ca^{2+} concentration medium sets up slowlier than the one in a high concentration K^+ medium; nor does it reach a high amplitude even when the external Ca^{2+} is removed. In exchange, the repolarization of the membrane when remaking the $K^+ : Ca^{2+}$ normal ratio takes place very rapidly.

Both types of depolarization are a response of the membrane to the $K^+ : Ca^{2+}$ ratio increase, but in the former case its growth resulted from the increase of K^+ concentration and in the latter from the decrease of Ca^{2+} concentration. Though the two responses indicate that generally speaking, the membrane responds in the same way to the increase of the ratio concentrations of the two ions (by depolarization), their qualitative difference points to the specific way of reacting due to the specific means by which the result has been obtained by either the growth in K^+ concentration or the diminishing of Ca^{2+} concentration.

The comparison of the different ways of membrane repolarization in the two cases indicates that the phenomenon takes place either rapidly (if it depends on the concentration increase of extracellular Ca^{2+} after the depolarization in low Ca^{2+} concentration) or slowly (if it depends on the lowering in K^+ concentration, after the depolarization in a high K^+ concentration medium). In both cases there is, after all, a reduction of the $K^+ : Ca^{2+}$ ratio up to value of the normal resting state.

Similar observations can be made in the case of the two forms of hyperpolarization of the membrane, which are both the outcome of the reduction of the extracellular $K^+ : Ca^{2+}$ ratio. When the growth of the membrane potential results from the increase in concentration of external Ca^{2+}, it sets up rapidly and can be of relatively high amplitude (when Ca^{2+} is highly increased in concentration). If hyperpolarization results from the lowering in concentration of external K^+, it sets up slowlier nor does it reach a high amplitude even when the ion is wholly eliminated from the medium.

The repolarization occurring when hyperpolarized membranes are introduced in normal media follow the same rule. If K^+ is increased in concentration up to the normal value for restoring the normal membrane potential, the phenomenon takes place rapidly. If the Ca^{2+} concentration has to be lowered up to the normal value, the phenomenon evoluates much slowlier.

In both cases the repolarizations correspond to an increase of the $K^+ : Ca^{2+}$ ratio.

It has previously been shown that both the laminar micellae and the globular ones are different in what concern the degree of sensitivity to-

wards the ion with specific transitional action (Ca^{2+} in the former case and K^+ in the latter one). There is a very big number of micellae of a given type which are very sensitive toward the destructuring ion and to which the structuring specific ion is very weakly linked, and an increasingly smaller number of micellae with a steadily lower sensitivity toward the action of the transitional ion and to which the structuring ion is strongly linked.

Consequently, if an increased concentration of the destructuring ion is introduced in the medium (K^+ for the laminar micellae and Ca^{2+} for the globular ones), a great number of micellae will fastly undergo the phase transition, i.e. all those with respect to whose sensitivity the concentration of the destructuring ion is higher or at least equal to that necessary for producing the transition.

This occurs in depolarization when the K^+ concentration is increased or in hyperpolarization when Ca^{2+} concentration is increased. The micellar structures are affected from the ones with high sensitivity to the increasingly fewer ones, whose sensitivity tends to the limiting value, corresponding to the concentration of the specific agent. Micellae which hold the structuring ion strongly linked are not implied in the phase transition.

If a membrane is introduced into a medium with a low concentration of the structuring ion (Ca^{2+} for laminar micellae and K^+ for the globular ones), the first micellar structures to be affected by this change are the ones to which the structuring ion was the most strongly linked in its normal concentration now tending to lower and which are very few. It is only these ones that can be involved from the very beginning in the phase transition by the relatively increased concentration of the transitional ion (remained at the absolute value, characteristic of the normal resting state). Only when the concentration of the structuring ion steadily lowers, the micellae with high sensitivity toward the transitional ion, which are more and more numerous, can be affected. The direction in which the structuring ion concentration varies (it lowers from the normal value to smaller ones) and the relative growth in concentration of the destructuring ion lead to a slow development of phenomena, on the background of the differentiation between micellar structures. This occurs in the depolarization and hyperpolarization with a lowered concentration of Ca^{2+} and K^+, respectively.

Since the normal concentrations of the structuring ions in the extracellular medium are relatively small and can be lowered only up to zero, it is clear that these phenomena cannot reach high amplitudes.

The differentiation of repolarization, following the two forms of depolarization and hyperpolarization respectively, can be explained on this basis.

It must be taken into account that the membranes depolarized (one way or another) have an increased number of globular micellae in the outer layer, corresponding to the relatively or absolutely increased concentration of K^+ which is their structuring ion. When these membranes are reintroduced in a medium with a normal $K^+ : Ca^{2+}$ ratio, in the first case the normal concentration value of the structuring ion (K^+) of the initial state lowers effectively to the normal value, and the con-

centration of the destructuring ion (Ca^{2+}) of this state gets relatively higher. In the second case, the concentration of the destructuring ion (Ca^{2+}) of the initial state rises effectively to normal value and the concentration of the structuring ion (K^+) of this state gets relatively lower. The phenomenon is slow here, as well, when it has at the basis the reduction of the concentration of the structuring ion of the affected state, and is fast when it is triggered by the growth of the concentration of the destructuring ion of this state.

In the case of hyperpolarized membranes (of both types) we have to deal with an increased number of laminar micellae in the external layer, corresponding to the absolutely or relatively increased concentration of Ca^{2+}, which is the structuring ion in this case. When introducing the membranes in a medium in which there is a normal $K^+ : Ca^{2+}$ ratio, the membrane potential is restored rapidly when the concentration of the destructuring ion of the initial state is increased, and slowly when the concentration of the structuring ion of the state is lowered.

Thus, the following general remarks can be made :

— At the basis of the reduction of the membrane potential (depolarization, or restoration of membrane potential to the initial state, after hyperpolarization), which involves the external $K^+ : Ca^{2+}$ ratio, lies the increase in value of this ratio.

— Any increase in the membrane potential (hyperpolarization, or restoration of membrane potential to the initial state, after depolarization), which implies the external $K^+ : Ca^{2+}$ ratio, is triggered by the lowering in value of the latter.

— The variations of potential discussed, in comparison with the micellar structures involved in the phase transition which causes them, take place very rapidly if they result from a growth in the concentration of the specific destructuring ion, and very slowly when the concentration of the structuring specific ion is lowered.

— The variations in potential analysed here can have great amplitudes when resulting from the increase in concentration of the destructuring ion, and only small amplitudes when resulting from the lowering in concentration of the structuring ion.

REFERENCES

1. Adrian R. H., 1956, J. Physiol., **133**, 631.
2. Agrigoroaei Şt., 1974, *Cercetări asupra mecanismelor biofizice ale proceselor de excitaţie şi permeaţie la nivelul organizării moleculare a membranelor fibrelor excitabile.* Thesis, Iaşi.
3. Agrigoroaei Şt., Agrigoroaei G., 1980, Rev. Roum. Biol.—Biol. Anim., **25** (*2*).
4. Crăciun V., Agrigoroaei Şt., 1978, Rev. Roum. Biol.—Biol. Anim., **23**, (*2*), 139.
5. Curtis B. A., 1963, J. Physiol., **166**, 75.

6. Frank G. B., Inoune F., 1973, Jap. J. Physiol., **23**, 183.
7. Hodgkin A. L., Horowicz P., 1959, J. Physiol. (London), **148**, 127.
8. Jenerick H. P., Gerard R. W., 1953, J. Cell. comp. Physiol., **42**, 79.
9. Jenerick H. P., 1956, J. Gen. Physiol., **39**, 773.
10. Kolb H. A., Adam G., 1976, J. Membrane Biol., **26**, 121.
11. Lassen U. V., Pape L., Vestergaard-Bogind B., 1976, J. membrane Biol., **26**, 51.
12. Neacşu I., Agrigoroaei Şt., 1980, Rev. Roum. Biol—.Biol. Anim., **25** (*1*).
13. Neacşu I., Agrigoroaei Şt., 1978, Rev. Roum. Biol.—Biol. Anim. **23** (*2*), 133.
14. Shanes A. M., 1958, Pharmacol. Rev., **10**, 58.
15. Straub R., 1956, Arch. Int. Pharmacodyn., **57**, 414.

Received February 15, 1980

Biological Research Center,
Iaşi, 6600, *Calea* 23 *August no.* 20 *A*

INSULIN AND ($Na^+ - K^+$)-PUMP

BY

MARGARETA CRĂCIUN, ŞT. AGRIGOROAEI and P. JITARIU

Our experiments were performed on single muscle fibres, using the intracellular glass microelectrode technique. In the absence of external K^+ although insulin binds to its specific membrane receptors it stimulates no more the active ionic transport. Moreover it inhibits the passive hyperpolarization brought about by the lack of K^+. If the phase transition of the phospholipidic micella (globular to laminar) already took place the hormone was without effect. The absence of external sodium completely prevented the insulin binding to membrane and, as a result, any bioelectric effect of the hormone. These results support the hypothesis of insulin stimulation of the ($Na^+ - K^+$)-pump.

The first observations with respect to the bioelectrical effects of insulin were made by Zierler [18]—[20] which explained the hyperpolarization in the presence of the hormone as a result of K^+ accumulation in the muscle fibres. Subsequently, this assumption was not confirmed by the experimental observations made by others. Thus, in 1972 Moore [14] proved that insulin acts first on the active Na^+ efflux but the modifications of the K^+ influx and, consequently, the RP increase are auxiliary. In addition, Beigelman [6] considers that the modifications and the transport of amino acids, monosugars and ions determined by insulin are secondarily related to the primary effect on the cell manifested by the RP increase. The author suggested even that in these conditions the membrane can behave as "bioelectric generator" according to the mechanism suggested by Grundfest [12].

The primary mechanism of the insulin action on the cell membrane being so controversial, our experiments tried to elucidate first some aspects yet unclearified.

A series of our already published results [7] support the hypothesis of stimulation by insulin of the $Na^+ - K^+$ active transport. In this paper we add some supplementary data supporting this view.

MATERIAL AND METHODS

Our experiments were performed on single frog sartorius muscle fibres using the intracellular glass microelectrode technique and an electronic Tönnies device for amplification and recording. A Ringer bicarbonate buffer (pH = 7.2) was used throughout the experiments. Insulin

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, No 2, P. 171—176, BUCAREST, 1981

solutions (0.05 I.U./ml) were prepared by adding corresponding amounts of hormone (Biofarm, 40 I.U./ml) to the Ringer solution. The K^+-free Ringer solutions were obtained by equimolar substitution of Na^+ for K^+. The choline solution was prepared by the 100 % replacement of Na^+ (113.9 mM) by Cl—choline.

All experiments were carried out at room temperature (22—25°C) on groups of 5—6—sartorii. The statistical significance of the observed changes for each experimental series was evaluated by Student's test.

RESULTS

1. *The insulin effect in the absence of external K^+.* A first series of observations were made on fibres upon which the hormone acted from the start of the experiment in the K^+-free medium. If the K^+-free Ringer caused a mean hyperpolarization of 14.3 mV within an hour (Fig. 1A), the same solution, but containing 0.05 I.U./ml insulin, brings about only a mean RP increase of 4.8 mV within the same time (Fig. 1B). Significant

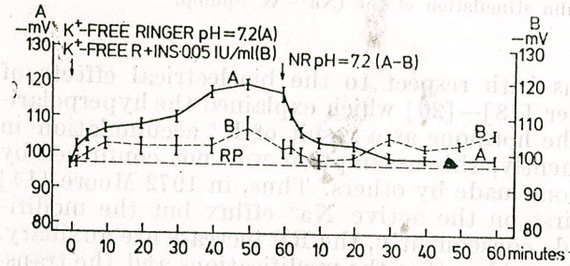


Fig. 1. — The insulin effect on the RP in the absence of external K^+ (B) and membrane hyperpolarization in the absence of external K^+(A).

differences were found also when the fibres were brought back into normal Ringer. The membranes hyperpolarized by the lack of external K^+ were repolarized almost completely within 30 minutes. However, those pretreated as well with insulin in K^+-free Ringer exhibited a tendency of the potential to return within the first 10—20 minutes, after which they became hyperpolarized again. After 60 minutes in hormone-free Ringer, the potential increase was 7 mV which proves that insulin binds to its specific membranal receptors even in the absence of K^+ but acts on the membrane only after the normal ionic ratios were reestablished.

The same concentration of insulin (0.05 I.U./ml), but acting from the beginning in normal Ringer, causes a mean potential increase of 9—10 mV within an hour (Fig. 2A) as compared to the hyperpolarization of

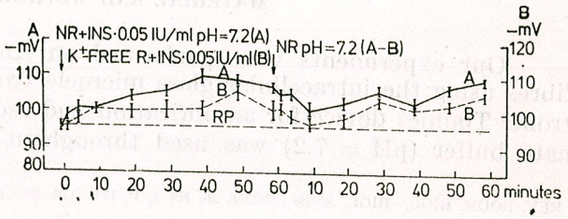


Fig. 2. — The insulin effect on the RP in normal Ringer A) and in K^+-free Ringer (B).

only 4.8 mV caused by the lack of external K^+ during the same time (Fig. 2B). By bringing the fibres back to normal Ringer, the hyperpolarization is maintained in both fibre lots but, in those treated with insulin in the lack of external K^+ the phenomenon was less ample and took place after an initial return to the reference value.

A completely changed situation occurs when insulin acted upon the already hyperpolarized membranes in K^+-free medium. In this case the hormone has no significant effect although it binds to the membrane. This results from the fact that after repeated washings with normal Ringer the insulin hyperpolarization lasted for a long time (Fig. 3B) whereas that caused by lack of external K^+ disappears within the first half hour (Fig. 3A).

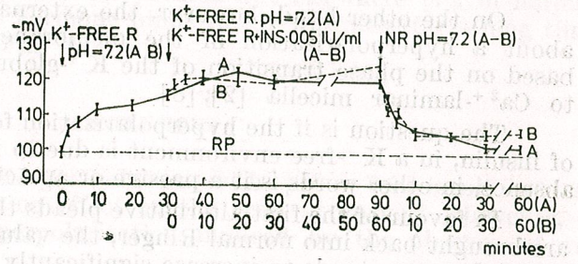


Fig. 3. — The insulin effect in the absence of external K^+ after a treatment with K^+-free Ringer (B) compared to the effect of K^+-free Ringer alone (A).

2. *The insulin effect in the absence of external Na^+.* The complete replacement of external Na^+ for Cl—choline deprives the pump of its specific substrate, the Na^+ passive influx being zero in this case. In these conditions, the insulin addition (0.05 I.U./ml) should have no stimulating effect in the pump and our experiments confirm this fact (Fig. 4B).

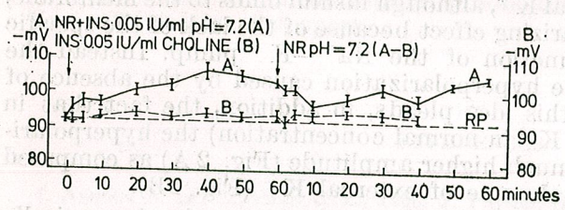


Fig. 4. — The insulin effect on RP in a Na^+-free Ringer solution (B) in comparison with its effect in a normal Ringer (A).

Moreover, in free-Na^+ medium, the hormone does not even bind to the membrane since no remanent hyperpolarization occurs which can be observed even after repeated and prolonged washings with normal Ringer when insulin is firmly bound to the membrane (Fig. 4 A).

DISCUSSIONS

Among the numerous hypotheses concerning the mechanisms of insulin action on the cell membrane, the one which puts the primary effect of the hormone on a stimulation of the Na^+—K^+ active transport seems to be the most justified.

By an accurate and adequate manipulation of the experimental conditions five different working ways of the pump were evidenced [11], namely : the $Na^+—K^+$ exchange (normal way) ; $K^+—Na^+$ exchange (reversed way) ; $Na^+—Na^+$; $K^+—K^+$; and uncoupled Na^+ efflux [10].

In the normally functioning mode of the pump (i.e. $Na^+—K^+$ exchange) ATP is required, the hydrolysis of each nucleotide molecule being accompanied by the efflux of $3Na^+$ and influx of $2K^+$ which result a net charge movement across membrane (i.e. the pump is electrogenic). The resulted hyperpolarization is sensitive both to the lack of oubain [7] and K^+ [1], [9], [17].

If we admit [3] that insulin stimulates just this type of active transport (i.e. electrogenic pump : $3Na^+/2K^+$) it could be expected that in a K^+-free solution the hormone effect is annulated or at least decreased.

On the other hand, however, the external K^+ absence itself brings about a hyperpolarization of the membrane (Fig.1A), a phenomenon based on the phase transition of the K^+-globular phospholipidic micella to Ca^{2+}-laminar micella [2], [8].

The question is if the hyperpolarization found by us in the presence of insulin, in a K^+-free environment is due to the hormone or to the K^+ absence, in other words is it a passive or an active RP increase ?

In favour of the first alternative pleads the fact that after the fibres are brought back into normal Ringer, the value of the potential, after an initial decrease, tends to increase significantly (Fig. 1B). That is a proof that insulin is firmly bound to the membrane, even in the absence of K^+ and so its effect is visible only when the ionic ratios were restored. But, against an active hyperpolarization due to insulin pleads in itself the fact that in normal Ringer, the initial phase is an almost complete return of the potential to the reference value ; thus the hormone effect occurs much later and does not represent a continuation of the previous phase. Therefore, in the absence of external K^+, although insulin binds to the membrane, it cannot exert its hyperpolarizing effect because of the lack of the specific substrate for the normal function of the $Na^+—K^+$ pump. Instead the hormone inhibits the passive hyperpolarization caused by the absence of external K^+. In favour of this idea pleads, in addition, the fact that in a physiologic solution (with K^+ in normal concentration) the hyperpolarization due to insulin has a much higher amplitude (Fig. 2 A) as compared with that observed in the absence of external K^+ (Fig. 2B).

However, if the phase transition of the globular to laminar micella due to the absence of external K^+, has already occurred being accompanied by the characteristic hyperpolarization, the subsequent addition of hormone has no effect, the amplitude of the hyperpolarization remaining unmodified (Fig. 3B). In these conditions too, insulin binds to its specific membrane receptors but acts on the active transport merely after the replacement of the K^+-free solution by a Ringer solution with normal ionic ratios.

When the external K^+ concentration is normal but Na^+ is lacking (by substitution with choline) it may be expected, again, that the $Na^+—K^+$ coupled active transport is inhibited. It is known that choline is a good substitute for sodium [13], [15], [16] and that it does not penetrate

through the membrane within the cell. Nastuk and Hodgkin [15] pointed out, however, that by the almost complete substitution of choline for Na^+, a weak reversible hyperpolarization in normal Ringer can be obtained. This increase of the potential can be justified by an active Na^+ efflux (sensitive to oubain), uncoupled with the influx of Na^+ or K^+. Such a way of pump functioning was evidenced with crab nerve [4] and frog muscle [5] incubated in K^+-free media.

In these conditions, the absence of any bioelectric effect of insulin may be admitted and our experiments have proved this. But in the lack of external Na^+ (substituted with choline) insulin not only has no effect in respect to the stimulation of active transport but it does not even bind to the membrane. This clearly can be seen from the fact that after the fibres were brought back into normal Ringer the value of the potential remains unmodified (Fig. 4B). As pointed out elsewhere [7], in normal Ringer at pH = 7.2, the hormone binds firmly and acts for a long time on the cell membrane (Fig. 4A).

CONCLUSIONS

1. In a K^+-free Ringer solution with pH = 7.2, insulin (0.005 I.U./ml) has no stimulating effect on the active transport. However, it inhibits the passive hyperpolarization induced by the absence of external K^+.

2. After the phase transition of the K^+-globular micella to Ca^{2+}-laminar micella has already occurred, insulin influences no more the effect of the external K^+ absence but it binds, however, to the cell membrane.

3. The total lack of external Na^+ prevents insulin from binding to the membrane and all the more so the stimulation of active transport by the hormone.

All these are incontestable proofs that insulin interacts with the $Na^+—K^+$ active transport.

REFERENCES

1. Adrian R. H., Slayman C. L., 1966, J. Physiol. (London), **184**, 970—1014.
2. Agrigoroaei Șt., 1974, *Cercetări asupra mecanismelor biofizice ale proceselor de excitație, și permeație la nivelul organizării moleculare a membranelor fibrelor excitabile*, Thesis, Iași.
3. Agrigoroaei Șt., Crăciun M., 1978, Rev. Roum. Biol.—Biol. Anim., **23**, 1, 63—67.
4. Baker P. F., 1964, Biochim., Biophys. Acta, **88**, 458—460.
5. Beaugé L. A. Ortiz O., 1972, J. Physiol. (London), **226**, 675—697.
6. Beigelman P. M., 1965, J. Amer. Diabetes Assoc., **14**, 8, 529—530.
7. Crăciun M., Agrigoroaei Șt., 1978, Rev. Roum. Biol.—Biol. Anim., **23**, *2*, 143.
8. Crăciun V., Agrigoroaei Șt., 1978, Rev. Roum. Biol.—Biol. Anim., **23**, *2*, 139.
9. Cross S. B., Keynes R. D., Rybova R., 1965, J. Physiol. (London), **181**, 865.
10. Glynn I. M., Karlish S. J. D., 1974, In : *Membrane Adenosine Triphosphatases and Transport Processes*, Ed. R. Bronk, London, Biochem. Soc.
11. Glynn I. M., Karlish S. J. D., 1975, Ann. Rev. Physiol., **37**, 13—55.
12. Grundfest H., 1953, In : *Electrochemistry in Biology and Medicine*, Ed. T. Shedlovsky Wiley, New York.

13. Lorente de Nó R., 1949, J. Cell. Comp. Physiol., **33**, Suppl. **1**.
14. Moore R. D., 1972, J. Physiol., **232**, 1, 23—45.
15. Nastuk W. L., Hodgkin A. L., 1950, J. Cell. Comp. Physiol., **35**, 39.
16. Shanes A. M., 1958, Pharmacol Rev., **10**, 58.
17. Thomas R. C., 1972, Physiol. Rev., **52**, 563—594.
18. Zierler K. L., 1957, Science, **126**, 1067.
19. Zierler K. L., 1959 a, Amer. J. Physiol., **197**, 3, 515—523.
20. Zierler K. L., 1959 b, Amer. J. Physiol., **197**, 3, 524—526.

Received April 10, 1980

Biological Research Center
Iași, 6600, Calea 23 August no. 20 A

ON SOME ECTOPARASITES OF *MICROTUS ARVALIS* PALLAS AND *M. AGRESTIS* L. IN THE AGROSYSTEMS OF THE BRAȘOV DEPRESSION (ROMANIA)

BY

MARIA SUCIU and AL. TUȚĂ

In this paper the authors analyse the ectoparasites associates on *Microtus arvalis* and *Microtus agrestis* from agrosystems of the Brașov depression.

Complex and long-standing studies on the role of rodents from agrosystems were initiated within the Mammalogy Laboratory of the Institute for Plant Protection. As a result, a valuable material of ectoparasites of two species, *Microtus arvalis* Pallas and *M.agrestis* L., frequently met in our fauna and especially in the area which interests us, has become available.

The Brașov depression is the largest intraCarpathian depression (about 1800 km²) with an average altitude of 550 m. It is divided into three main parts: the Bîrsa depression (Țara Bîrsei) in the West, the Șesu Frumos plain in the middle and the Trei Scaune depression in the East. The whole area is covered by agricultural crops.

MATERIAL AND METHOD

Rodents were collected between 1968 and 1971 from clover, lucerne, sedge and hay fields. Out of the 394 specimens of *Microtus arvalis* captured, 56 had ectoparasites and 13 out of the 35 specimens of *M. agrestis* were infested. Use was made of spring traps and hosts were killed and deparasitized after a longer time interval. Since the number of ectoparasites was relatively low, only approximate estimations as concerns density and frequency were possible. Twenty-one species and subspecies of acarians, lice and fleas were identified (Table 1).

RESULTS AND DISCUSSIONS

The situation of ectoparasites from *Microtus arvalis* and *M. agrestis* is the following.

Cyrtolaelaps mucronatus (G. et R. Canestrini). Predatory gamaside, common in the nests of rodents and insectivores, under stones, leaves or in various ratting substances. Deutonymphs found both on the body and in nests of rodents. Our material was collected from *Microtus arvalis* in clover and sedge crops. Associations were noticed with *Laelaps agilis*, *Eulaelaps stabularis* and *Hypoaspis* sp.

Table 1

Relations between parasites and hosts

No. crt.	Parasites	Number of specimens	Places of collecting	Years	*M. arvalis*	*M. agrestis*
	Acarians					
1	*Cyrtolaelaps mucronatus*	8	Măgurele	1969	+	
2	*Hamogamasus nidi*	32	Măgurele	1968	+	
			Lunca-Cîlnicului	1970		+
			Prejmer	1971		
			Pirușca			
3	*Hypoaspis* sp.	12	Măgurele	1968	+	
			Pirușca	1969		+
4	*Androlaelaps fahrenholzi*	57	Măgurele	1968	+	
			Covasna	1969		+
			L. Cîlnicului			
			Pirușca			
5	*Eulaelaps stabularis*	5	Brădet			
			Măgurele	1968	+	
			Brădet			+
			Pirușca			
6	*Myonyssus* sp.	1	L. Cîlnicului	1968	+	
7	*Laelaps hilaris*	60	Măgurele	1968	+	
			Hărman	1970		+
			L. Cîlnicului			
			Brădet			
			Prejmer			
			Pirușca			
8	*Laelaps agilis*	73	Măgurele	1968	+	
			Brădet	1969		+
			Codlea	1971		
			L. Cîlnicului			
			Prejmer			
			Pirușca			
9	*Hirstionyssus isabelinus*	12	Codlea	1968	+	
			Prejmer	1969		
			Măgurele			
10	*Hirstionyssus latiscutatus*	1	Măgurele	1968	+	
11	*Ixodes* sp.	9	Codlea	1968	+	
12	*Dermacentor* (D) *marginatus*	10	Hărman	1968	+	
	Lice					
13	*Hoplopleura acanthopus*	24	Măgurele	1968	+	
			Brădet			+
			Prejmer			
			Pirușca			
	Fleas					
14	*Hystrichopsylla talpae orientalis*	3	Pirușca	1968	+	
15	*Rhadinopsylla isacantha*	1	Măgurele	1968	+	
16	*Ctenophthalmus agyrtes romanicus*	14	Brașov	1968	+	
			Covasna			+
			Pirușca			
17	*C. assimilis assimilis*	28	Măgurele	1968	+	
			Codlea			
			Prejmer			
18	*Peromyscopsylla silvatica*	1	Măgurele	1971	+	
19	*Malaraeus penicilliger kratochvili*	2	Măgurel	1971	+	
		1	Pirușca	1968		+
20	*Megabothris turbidus*	2	Pirușca	1968		+

It is a holarctic species mentioned from the West of Europe up to Asia. In Romania it was also mentioned by L. Solomon [3] in the Călimani Mts, Livada and Ieșelnița.

Haemogamasus nidi Michael. It is a facultative ectoparasite, feeding on the blood from scratches on host skin or from fleas prickings. Its size greatly varies with the various stages of growth (adults average 0.93/0.82 mm). Their growth especially occurs in the nests and burrows of rodents. Females particularly occur on hosts. Parasitocoenoses observed by us on *Microtus arvalis* were mainly formed of acarians *(H. nidi, Laelaps agilis, L. hilaris, Androlaelaps fahrenholzi, Hirstionysus isabelinus, Myonysus* sp.), lice *(Hoplopleura acanthopus)* and fleas *(Hystrichopsylla talpae orientalis, Ctenophthalmus agyrtes romanicus, Malaraeus penicilliger kratochvili, Megabothris turbidus)*.

Haemogamasus nidi has a wide range of hosts : insectivores, rodents, carnivores. Sometimes it occurs in bird nests or in the wastes of beehives. Holarctic species. It is widely spread in Romania.

Hypoaspis sp. It belongs to a complex genus with an insufficiently elucidated systematics. It infests insectivores and rodents to the same extent. Based on L. Solomon's accounts [3] we think that Hypoaspis's area in Romania is wide.

Androlaelaps fahrenholzi Berlese. A species with weak parasitic specificity. It infests small mammals and even birds. It may be found also on men. It is of epidemiologic interest (tularemia, hemorrhagic nephrites).

In our cases, parasitocoenoses on *Microtus arvalis* were made of acarians *(A. fahrenholzi, Hypoaspis* sp., *H. nidi. E. stabularis, L. hilaris, L. agilis, Hirstionyssus latiscutats)*; *lice (H. acanthopus)* and fleas *(C. agyrtes romanicus, C. assimilis assimilis, Peromyscopsylla silvatica, M. penicilliger kratochvili)*. Those on *M. agrestis* consisted of acarians *(H. nidi, Hypoaspis, L. hilaris, L. agilis)*; lice *(H. acanthopus)* and fleas *(H. talpae orientalis)*. Frequent associations occur with *L. agilis* and *L. hilaris*.

It is a cosmopolitan species widely spread in Romania [4].

Eulaelaps stabularis (Koch). It is a common species from rodents. Literature mentions it on 42 species of hosts from various orders of mammals and birds. The males, larvae, protonymphs and deutonymphs live in the nest and burrows of rodents. Females are more often met on rodents and sometimes enter human dwellings. We have found this species associated with other acarians *(L. hilaris* and *L. agilis, A. fahrenholzi, H. nidi)*; lice *(H. acanthopus)* and fleas *(C. assimilis)*.

It is an ubiquous species, with a wide area in Romania.

Myonyssus sp. The species of this genus are parasites on insectivores and rodents. In Romania they are little mentioned [2], [3].

Laelaps hilaris Koch. A common parasite of small mammals, with specificity for *M. arvalis*. It is one of the most frequently identified species on this host. Its association includes : other acarians *(H. nidi, Hirstionyssus* sp., *A. fahrenholzi, Hypoaspis* sp.); lice *(H. acanthopus)* and fleas *(C. agyrtes romanicus, P. silvatica, M. penicilliger Kratochvili)*. Most often met together with *A. fahrenholzi*.

L. hilaris is an Euroasian species widely spread in Romania.

Laelaps agilis Koch. It is specific to *Apodemus sylvaticus* but it is also met on other species of rodents and on insectivores. All preadult stages and embryonated females occur in the fur of hosts.

It is common in the studied area. Prevailing associations with other acarians are those with *H. nidi* and *A. fahrenholzi*.

Holarctic species. Its area coincides with that of the main host (*Apodemus sylvaticus*).

Hirstionyssus isabellinus (Oudemans). Compulsory parasite, with a wide range of hosts among insectivores and rodents. Holarctic species. In Romania it was mentioned in various regions [3].

Hirstionyssus latiscutatus (de Meillon et Lavoipierre). Haematophagous species, parasite of rodents. Cosmopolitan.

Ixodes sp. Only nymphs from *M. arvalis*.

Dermacentor (Dermacentor) marginatus (Sulzer). Adults are parasites of domestic animals and of some wild ones. They also met on small mammals (insectivores, rodents and carnivores) and on hoofed animals, sometimes on birds. It is of higher epidemiologic interest as vector of *Babesia* and various viruses.

The species is widely spread and prefers less dry regions of East and South Europe and Central Asia.

Hoplopleura acanthopus (Burmeister). According to J. C. Beaucournu [1] *Microtus arvalis* is the main host of this species. *H. acanthopus* was recorded in Romania by T. Wegner [8] on various insectivores and rodents of Dobrogea and by M. Voicu [6], [7] in Moldova and Muntenia. From all these mentions and from our observations there results that *H. acanthopus* is a common parasite on many species of rodents in Romania and especially on *Microtus arvalis*. The latter infests all components of the populations in a biocoenosis.

Holarctic species. In Romania it seems to be one of the species with the widest area.

Hystrichopsylla talpae orientalis Smit. The mole flea infests especially small mammals in the hill and mountain forests and it has no rigorous parasitic specificity. It is widespread in Europe and Asia (U.S.S.R.).

Rhadinopsylla isacantha (Rothschild). It is a rare species in our country. European flea.

Ctenophthalmus agyrtes romanicus Suciu. This species is especially parasite of *Apodemus*. It is also met on other small mammals living in the same biotope as the main host. It is spread on a vertical line from lowland areas to mountain ones.

Ctenophthalmus assimilis assimilis (Taschenberg). It is a nest flea, found often enough in the fur of many small mammals, from various biotopes. European species, widespread in Romania.

Peromyscopsylla silvatica silvatica (Meinert). It infests small mammals from high altitude biotopes. Species characteristic of Boreal Europe, of the European part of the U.S.S.R. and of the Ural. In Romania, it is often met in the Carpathian zone.

Malaraeus penicilliger kratochvili Rosický. It is parasite of the *Microtidae*. This species is widespread in the alpine areas of Europe. In Romania it was mentioned in various massifs [5].

Megabothris turbidus (Rothschild). Flea which often infests mice of the *Apodemus* genus as well a other rodents. It is spread in Europe, Asia and Asia Minor. In Romania it is variously distributed on a vertical line, from the area of subcarpathian hills to the area of mountain woods, not exceeding it.

Our field observations and collectings led us to the conclusion that the structure of rodent populations as well as the number of specimens forming them vary every year. The situation of *Microtus arvalis* is the following : 183 specimens were captured in 1968, 76 in 1969, 57 in 1970 and 78 in 1971. Within two years (1968, 1969), a number of 21 and 14 specimens of *M. agrestis*, respectively, were captured. Depending on host variations, a specific and numeric variation of the ectoparasites is noticed. Twelve species of acarians, one species of lice and seven species and subspecies of fleas were identified. Both *M. arvalis* and *M. agrestis* are infested especially by acarians.

The most common species of acarians on *M. arvalis* are *Androlaelaps fahrenholzi*, on 21 specimens of hosts, *Laelaps hilaris* on 18 hosts and *Haemogamasus nidi* on 13. In the case of *M. agrestis*, two species of acarians are common : *Laelaps agilis* on 7 specimens of hosts and *H. nidi* on 16.

Hoplopleura acanthopus also confirms its preference for *M. arvalis* which is considered the main host. This species is common enough on *M. agrestis*.

Fleas are well represented in the biotopes studied by *Ctenophthalmus agyrtes romanicus* and *C. assimilis assimilis*. We mention that *Peromyscopsylla silvatica silvatica* and *Malaraeus penicilliger kratochvili* were identified out of those that prefer high altitude biotopes. They were collected in the Măgurele research station (Brașov).

Frequent associations were made between *Laelaptidae* species. In few cases, species of acarians, anoplura and siphonaptera were associated.

The parasitic specificity of the species included in this paper is weakly expressed. Most of them have a wide range of rodents and insectivore hosts. In general, the populations of *M. arvalis* and *M. agrestis* of the same biotopes have the same parasitofauna.

A first analysis of the complex of ectoparasites on the two species of *Microtus* in the studied agrosystems pointed out that the presence of species of acarians, lice and fleas, the number of specimens and the found-ed associations did not result in disturbances in the rodent populations, which are in a few number of specimens in the agrosystems.

REFERENCES

1. Baeucournu J. C., 1968, Ann. Parasit. Hum. et Comp., **43**, 2, 201–271.
2. Negoescu Ileana, 1976, Tr. Mus. Hist. Nat. "Gr. Antipa", **17**, 31–38.
3. Solomon Libertina, 1970, Rez. thesis.
4. Solomon Libertina, 1971, Com. Inst. Ped. Univ. "Al. I. Cuza" Iași, 295–318.
5. Suciu Maria, 1973, St. Com. Mus. St. Nat. Bacău, **6**, 47–72.
6. Voicu M., 1970, Lucr. Ses. Șt. Ocrt. Nat. Suceava, **6**, 157–161.
7. Voicu M., Cazacu Catinca, 1970, St. Com. St. Nat. Suceava, 103–106.
8. Wegner Zofia, 1970, Com. Zool. Soc. St. Biol. R.S.R., 305–314.

Received March 12, 1980

Faculty of Biology
Bucharest, Splaiul Independenței 91–95.

WITTENBERGEN CAROL, *Eseuri de biologie teoretică* (Essais de biologie théorique), 1 vol. 267 pages, Ed. ştiinţifică şi enciclopedică, Bucarest, 1981.

Je suis convaincu que chaque chercheur qui travaille dans un des domaines de la biologie sent, de temps en temps, un besoin intrinsèque de s'arrêter un moment et de méditer profondément sur les buts et les conséquences de son travail. Un tel arrêt, on le fait difficilement tout seul, car on a besoin d'une méditation philosophique, qui recquiert la connaissance de certaines notions ou données de cette discipline pour les utiliser à comprendre les problèmes de la biologie.

Le livre de Wittenberger est absolument nécessaire dans un tel arrêt, car il répond exactement aux besoins de notre question. Ce livre est une méditation compétente d'un biologiste sur les concepts philosophiques et d'un philosophe sur les problèmes biologiques, l'auteur étant aussi bien l'un que l'autre, mais surtout un excellent biologiste. Cet avantage lui permet de travailler en même temps avec les concepts philosophiques et avec ceux biologiques, sans faire ni de l'un ni de l'autre un excès terminologique. C'est pourquoi le livre se lit avec un très grand intérêt. On apprend comme toute naturelle l'immixtion de la philosophie dans la biologie et l'inverse ainsi que le fait que ces deux domaines ne peuvent être séparés. Mais au cours du temps ces deux disciplines ont été rarement parallèles, quelquefois une était en avant, autrefois l'autre, et il y avait des moments où leur interpénétration était très profonde et on ne pouvait pas les séparer.

La biologie, l'un des domaines complexes de la connaissance humaine, pose de multiples problèmes de différenciation entre de diverses disciplines, chacune avec son histoire, avec ses liaisons, avec sa quantité de données spécifiques, etc. Il s'impose surtout la mathématisation de ces données, non seulement pour mieux pouvoir connaitre les lois biologiques, mais surtout pour un changement de la conception qualitative en une conception quantitative des phénomènes biologiques.

Ces différents aspects des problèmes de la biologie constituent les fondements sur lesquels on peut étudier « cette forme de mouvement de la matière, qui s'appelle la Vie ».

La Vie doit d'abord être définie pour « *savoir de quoi on parle* », une question très discutée, mais absolument possible si on arrive à préciser ses caractères quantitativement spécifiques. Mais on ne peut pas séparer le phénomène de la Vie des autres formes de mouvement de la matière.

La Vie existe en individus concrets et ceux-ci présentent des « mouvements » (en sens philosophique) déterminés par des causes cognitives. On discute dans ce livre sur le causal et le hasard, sur la finalité et la liberté, sur les dimension spatio-temporelles et sur la qualité de la vie.

Une grande partie du livre est consacrée à *l'évolution* et à ses caractéristiques (réactivité, énergétique, autonomie) avec leurs tendances constantes vers la perfection. Dans ce cadre on s'occupe de l'évolution de l'homme et de sa position au sommet de l'hélicoïde, de la valeur cosmique de celui-ci et de l'importance de son cerveau pour l'univers aujourd'hui connu. Il y a aussi une très intéressante discussion sur l'avenir de l'homme dans son milieu actuel, où tous les paramètres de son existence prennent des vitesses de plus en plus grandes.

Il est très difficile de présenter dans quelques lignes la richesse extraordinaire des idées qui se rapportent à la Vie et à son évolution en général et celle de l'homme en spécial. L'auteur est convaincu qu'on doit regarder avec confiance l'avenir, car *la pensée*, phénomène unique dans le cosmos actuellement connu, qui se réalise avec une quantité infime d'énergie, doit surpasser et résoudre les problèmes sociaux et mondiaux qui se font avec des quantités énormes d'énergie. L'homme doit et peut chercher lui-même la voie de son futur et trouver les résolutions qui peuvent être appliquées pour que son maintien sur la Terre soit assuré.

Je pense que le livre de Carol Wittenberger devrait être traduit dans une langue internationale.

En tout cas, je recommande avec la plus grande conviction à tous ceux qui s'occupent de la Vie : biologistes, médecins, agronomes, philosophes, artistes et même politiciens, de le lire et de méditer profondément sur le sens général de la Vie et sur les destins de l'homme actuel et futur.

Eugen A. Pora

REV. ROUM. BIOL.-BIOL. ANIM., TOME 26, N° 2, P. 185–186, BUCAREST, 1981

IOAN I. BĂRA, GOGU I. GHIORGHIȚĂ, *Din enigmele evoluției* (Enigmes de l'évolution). Ed. științifică și enciclopedică, Bucarest, 1980, 215 p., 11 fig.

Ainsi que nous indique le sous-titre de ce livre, les auteurs se proposent d'y envisager l'apomixie et son rôle dans l'évolution, notamment celle des végétaux, vu leur domaine de recherches. Les trois premiers chapitres retracent successivement l'historique des théories évolutionnistes, les preuves de l'évolution et les facteurs déterminant les processus évolutifs. Après avoir rappelé l'évolution des modes de reproduction dans la règne végétal, les auteurs nous offrent un chapitre approfondi sur l'apomixie, y analysant ses modalités chez les plantes et ses implications évolutives au niveau des populations et des espèces. Malgré les difficultés théoriques qu'elles soulèvent par rapport aux concepts généraux reposant sur l'étude des espèces biparentales, il est à conclure qu'en raison de leur validité biologique et de leur capacité de stabiliser les effets du hétérosis, les formes apomictiques sont aptes à survivre dans des conditions peu favorables de milieu et peuvent devenir un matériel de choix pour la sélection et les activités d'amélioration des plantes de culture. C'est l'examen de ces perspectives qui forme l'objet du dernier chapitre, où à côté des possibilités d'hybridation et de polyploïdie, sont relevés les avantages des souches haploïdes pour la perpétuation des lignées homozygotes. Une bibliographie abondante complète ce volume qui est d'une lecture entraînante et à la fois utile à tous ceux intéressés de près aux problèmes généraux ou appliqués de la biologie.

Radu Codreanu

N. MANOLESCU (Edit.), *Citologie normală și patologică la animale* (Cytologie normale et pathologique des animaux domestiques). Ed. Ceres, Bucarest, 1980, 290 p., 63 pl. noir-blanc, 18 pl. couleurs.

Ce livre, issu de la collaboration étroite et compétente de 10 spécialistes, vise à nous offrir un ensemble de données cytologiques d'un intérêt actuel non seulement pour une meilleure compréhension de l'organisation morpho-fonctionnelle de nos animaux domestiques mais aussi pour les problèmes généraux de la biologie cellulaire normale et pathologique. Un premier aperçu de l'ultrastructure cellulaire et de ses principaux organites est suivi par un chapitre de cytogénétique nous renseignant sur les caryotypes du porc, cheval, bovin, ovin et de la chèvre (I. Voiculescu, Agripina Lungeanu). Les cultures cellulaires *in vitro* (C. Știrbu) sont ralliées à la détermination cytogénétique de certaines lignées celullaires et aux phénomènes de fusion cellulaire et de transfert chromosomique de gènes (I. Voiculescu). La transformation blastique des lymphocytes *in vitro* précède une étude des cellules mésenchymatiques de l'hématopoïèse normale médullaire, réticulo-lymphoplasmocytaire et périphérique, y compris la cytologie de la bourse de Fabricius des oiseaux (N. Manolescu, V. Ciocnitu). On y trouve un complément dans la cytopathologie du mésenchyme hématoformateur et dans la cytologie du tissu conjonctif (N. Manolescu, N. Avram). Font suite les chapitres consacrés aux aspects normaux et pathologiques des tissus chondro-osseux, musculaire et des structures utéro-ovariennes (N. Manolescu), de la cytologie hépatique, du parenchyme pulmonaire, des spermatozoïdes et du néphron (R. Begnescu), des glandes endocrines, du tractus gastro-intestinal (E. Bucur) et du tissu nerveux (Gh. Păunescu). Les deux derniers chapitres ont trait aux relations possibles entre chromosomes, virus et les proliférations malignes, telle la leucémie bovine (Agripina Lungeanu) ainsi qu'aux techniques d'immuno-fluorescence (D. Păltineanu). Des techniques de cytogénétique sont consignées dans un appendice final. Une riche illustration hors-texte rehausse l'attrait du volume qui s'adresse à des lecteurs de professions multiples : biologistes, médecins, zootechniciens, vétérinaires.

Radu Codreanu

REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

TOME 26 1981

INDEX ALPHABÉTIQUE

REV. ROUM. BIOL.—BIOL. ANIM., TOME 26, N° 2, P. 187—188, BUCAREST, 1981

AVIS AUX AUTEURS

La « Revue roumaine de biologie — Série de biologie animale » publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie animale : taxonomie, morphologie, physiologie, génétique, écologie, etc. Les sommaires des revues sont complétés aussi par d'autres rubriques, comme : 1. *La vie scientifique*, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. 2. *Comptes rendus* des livres de spécialité parus en Roumanie.

Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés à double interligne (31 lignes par page) en deux exemplaires.

La bibliographie, les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes exécutés à l'encre de Chine noire sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, les tableaux et les graphiques sera évitée. Les références bibliographiques, citées par ordre alphabétique des auteurs, comporteront le nom de l'auteur, l'initiale du prénom, l'année, le titre de la revue, abrégé conformément aux usances internationales, le tome, le numéro, la page.

Les travaux seront accompagnés d'un court résumé de 10 lignes au maximum, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

PRINTED IN ROMANIA