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SOMMAIRE

ANDY Z. LEHRER, *Sarcotakaops arnaudiella* Lehrer, 1985, est-elle en effet un synonyme de *Kanoa okazakii* (Kano, 1953)? (Diptera, Sarcophagidae) ... 3
ANDY Z. LEHRER, *Blackithiana* N. G., un nouveau genre paléarctique et la rédescription de son espèce-type *Blackithiana ornatijuxta* (Richet et al., 1995) (Diptera, Sarcophagidae) ... 11
PETRU M. BĂNĂRESCU, The status of some nominal genera of Eurasian Cyprinidae (Osteichthyes, Cypriniformes) ... 19
DORINA MIRANCEA, N. MIRANCEA, Dynamics of the cell-cell and cell-basement membrane junctions during skin ontogenesis ... 31
VIORICA MANOLACHE, VIRGINIA POPESCU-MARINESCU, MARIA NĂSTĂSESCU, CARMEN MARINESCU, Structural changes induced by copper intoxication in *Anodonta cygnaea* Linné *(Lamellibranchiata)* gills and hepatopancreas ... 41
VIORICA MANOLACHE, MARIANA ORĂŞANU, NICOLETA TELETIN, MARIANA GĂLEŞANU, VALERIA CLUCERESCU, DOINA PETRA-ŞINCU, LILIANA BABEŞ, Dynamics of the actin cytoskeleton in renal cells from the African green monkey (Vero) and in human pulmonary fibroblasts (ICP23 diploids) infected with measles virus ... 49
ANCA OANCEA, OANA CRĂCIUNESCU, MARIA CALOIANU, ŞT. MANTEA, MARIANA OPREA, The influence of oviductal conditioned medium on boar sperm motility and capacitation ... 57
MARIA CALOIANU, ANCA OANCEA, OTILIA ZĂRNESCU, Histochemistry and immunocytochemistry of glycosaminoglycans in sow oviduct ... 63
M. ISAC, D. E. CREANGĂ, R. M. ISAC, A statistical analysis on the *Drosophila melanogaster* eye electroretinogram ... 73
I. NEACŞU, C. CÂMPEANU, I. TRANDAFIRESCU, G. MUSTAŢĂ, Ionic tissular content of some mollusca species from the Romanian shore of the Black Sea ... 83
MARIA CIUBOTARIU, ANA DANIS, VIORICA MANOLACHE, GABRIELA DUMITRESCU, MARIA CUCU, E. I. IANCU, Some preliminary biomedical observations on U-contamination by ingestion in rats ... 89

OTILIA ZĂRNESCU, RADU MEŞTER, LUCIA MOLDOVAN, ANCA OANCEA, *In vivo* study of chondroitin sulphate uptake and distribution in oocytes of frog, *Rana ridibunda* 95
AL. G. MARINESCU, H. KÜNNEMANN, O. DRĂGHICI, LICA BARBU, S. ANDREUŢĂ , N. CRĂCIUN, Seasonal oxygen consumption in the goldfish *(Carassius auratus gibelio* Bloch.) 107
I. ROŞCA, FELICIA MUREŞAN, ANGELA UDREA, M. VOICU, V. BRUDEA, ELENA BUCUREAN, Researches regarding use of *Ostrinia nubilalis* synthetic sexual pheromone in Romania 117
IRINA TEODORESCU, A. VĂDINEANU, The pesticides pollution impact on the structure and dynamics of the arthropods association 125

COMPTE RENDU 137

SARCOTAKAOPS ARNAUDIELLA LEHRER, 1985, EST-ELLE EN EFFET UN SYNONYME DE *KANOA OKAZAKII* (KANO, 1953)? *(DIPTERA, SARCOPHAGIDAE)*

ANDY Z. LEHRER

A comparative study of the female characters demonstrates that the synonymy of the species *Sarcotakaops arnaudiella* Lehrer, 1985 and *Kanoa okazakii* (Kano, 1953) is a grave taxonomical error. These species are two distict and good palaearctical taxons.

En 1953, Kano a décrit l'espèce *Sarcophaga okazakii* pour la faune des Sarcophagides paléarctiques. Elle a été rédécrite et représentée dans la monographie ***Sarcophagidae*** de la **Fauna Japonica** (1967:84–86, fig. 47), étant très bien caractérisée par son armature génitale mâle. Sur ses femelles, qui sont sommairement décrites, Kano, Field & Shinonaga (supra, 1967:186) mentionnent que les «females of this species are easily differentiated from other related species by the extraordinarily broad palpi». Aussi, dans leur figure 47 (planche 26) sont figurés la forme des palpes (B_1) et des sternites postabdominaux VI–VII (E).

En 1985, nous avons décrit un genre nouveau et une espèce nouvelle sur la base d'une femelle capturée par J. L. Gressit dans les petits pois du mont Takao (qui a été localisé erronément par nous, à cause d'une ancienne toponymie, en Taiwan — d'après les renseignements reçus de dr. Etsuro Sugijama dans sa lettre du 10 juillet 1986). A la même occasion il a eu l'amabilité de nous offrir pour étude un mâle et une femelle de celle-ci, provenant de la collection du professeur R. Kano. Ayant ces exemplaires, nous avons pu faire la révision correcte de ces espèces et nous avons communiqué au dr. Sugijama notre conviction qu'elles sont de bonnes et distinctes espèces japonaises.

Surprenant est le fait que S. Shinonaga et T. Pape (1995) ont simulé une révision de ces deux espèces et ont «établi» une synonymie douteuse, fondée seulement sur deux arguments totalement non-scientifiques, en réitérant les impressions écrites par E. Sugijama, il y a 10 ans. Voila leurs arguments:

1) Ces auteurs ne connaissent que Kanoa okazakii qui a une femelle avec les palpes «gonflés». Ils disent: «***Sarcophaga okazakii*** **is the only species in the fauna of Japanes Sarcophagidae, and to the best of our knowledge in all of Oriental and Eastern Palaearctic fauna, where the femele palpus is distinctly swollen**» (supra, 1995:166). Mais, S. Shinonaga a oublié qu'il a écrit, avec les deux premiers coauteurs de la monographie de la **Fauna Japonica** (1967:73), que la femelle de l'espèce «shiriaka-nikubae» *(Parasarcophaga crassipalpis* Macquart) a les «**palpii thicker than that of the male**» et que dans la planche

99 des clés illustrées (1965) et dans la fig. 1 (la première planche colorée) de cette monographie ces palpes sont au moins deux fois plus larges que ceux du mâle.

2) Les deux espèces ont la même localité-type (Mont Takao), ce que ne constitue un argument valable ni pour les spécialistes débutants. Si elles ont été trovées dans la «**Tokyo Prefecture, Honshu**», il faut souligner à ces auteurs que cette «préfecture» a une longueur de presque 90 km et que le petit mont Takao se trouve — après leur mots — «**about 50 km west of the city of Tokio**». En plus, il n'a pas été spécifié parmi les localités où a été capturée *Kanoa okazakii* (cf. Kano, Field & Shinonaga, 1967:86), mais seulement dans le travail de Kano (1953).

En essayant de faire une comparaison entre les femelles de ces deux espèces en litige, nous avons constaté qu'elle est presque irréalisable. La cause principale consiste dans la non-concordance entre la description originaire de Kano (1953:258) et celle de la monographie des Sarcophagides de Japon (1967:85–86).

Ainsi, dans la première le front mesure 2/3 de la largeur d'un œil et les parafrontalies ont «**2 reclinate fronto-orbital bristles**», tandis que dans la seconde, le front mesure «**about four-fifths the width of one eye**» et les parafrontalies ont «**3 proclinate fronto-orbital bristles**». Pour les autres caractères somatiques on précise qu'ils sont similaires avec ceux du mâle.

Le manque des données sûres nous a déterminé à rédécrire la femelle de *Kanoa okazakii* d'après l'exemplaire donné par dr. E. Sugijama en 1986, dans le but de pouvoir confronter sûrement ses caractères avec la diagnose de *Sarcotakaops arnaudiella*. Aussi, nous avons sacrifié l'abdomen des deux espèces pour examiner comparativement leurs sternites abdominaux.

DESCRIPTION DE LA FEMELLE DE *KANOA OKAZAKII* (KANO, 1953)

Tête. Noire et couverte d'un tomentum argenté. Front, sur le vertex, mesure 2/3 de la largeur d'un œil. La bande frontale noire brunâtre est 1,5 fois plus large qu'une parafrontalie et dépourvu des fins macrochètes croisés. Les antennes sont noires à teinte légère brunâtre et avec la partie proximale du troisième article rougeâtre; les articles basaux sont d'un noir luisant; le troisième article est 2,5 fois plus long que le deuxième. Arista est noire brunâtre et avec de poils longs sur les deux parties. La trompe est noire à teinte brunâtre; les palpes très développés sous la forme d'une raquette de tennis (fig. 1), aplatis latéralement, un peu plus longs que le petit diamètre oculaire (45:40) et avec quelques fins microchètes sur le bord antéro-superterminal. Le péristome mesure un peu plus que 1/4 du grand diamètre oculaire.

Chétotaxie de la tête. Les macrochètes verticaux internes sont longs, forts et rétroclines; les macrochètes verticaux externes sont distincts et 1/2 de la

longueur des précédents; les ocellaires proclines et les préverticaux rétroclines sont bien développés et longs; les macrochètes frontaux sont au nombre de 10 paires (la dernière paire est rétrocline); il y a seulement 2 macrochètes para-

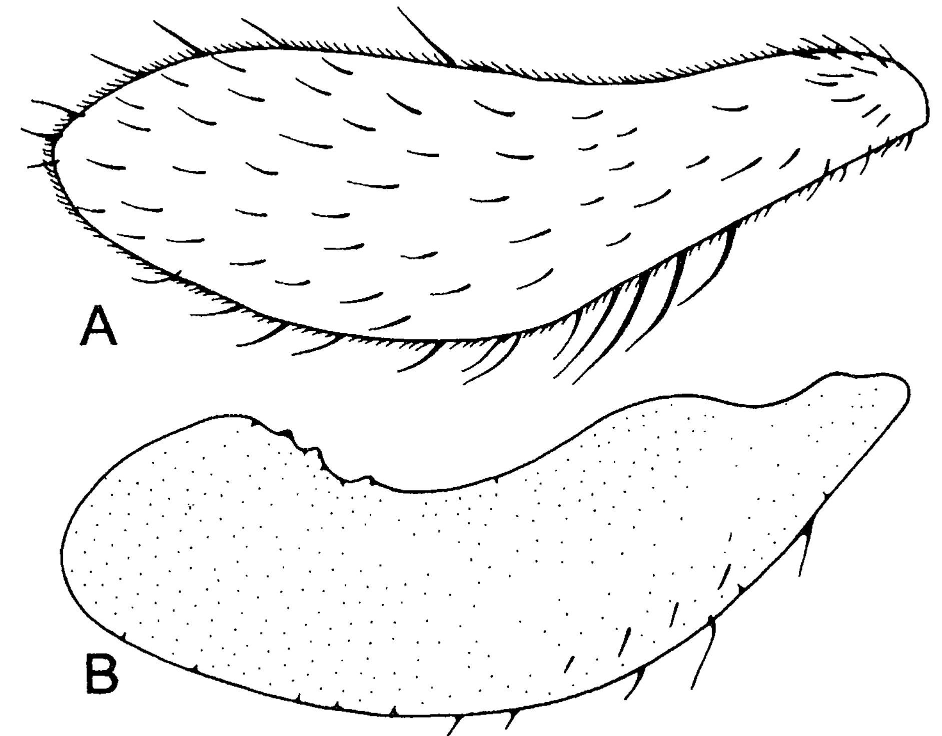

Fig. 1. — Palpes des femelles de *Kanoa okazakii* (Kano) (A) et de *Sarcotakaops arnaudiella* Lehrer (B).

frontaux (fronto-orbitaux) proclines sur chaque parafrontalie; 4–5 macrochètes parafaciaux assez longs se trouvent sur la marge antéro-inférieure de l'œil; les petites vibrisses montent jusqu'au milieu des bordures faciales; on observe 1 postocellaire et 1 postvertical sur chaque côté de l'occiput; les microchètes occipitaux sont disposés sur trois rangs irréguliers. Le péristome est couvert de poils noires; la partie potérieure de la tête a de poils blancs.

Thorax. Noir, avec tomentum argenté, trois bandes médio-dorsales longitudinales larges et deux bandes latérales étroites et courtes. Propleures glabres; prosternum poilu. Les stigmates sont brunâtres; ceux antérieurs plus noirâtres, ceux postérieurs plus rougeâtres. Scutellum a le bord postérieur plus ou moins droit. Les pattes sont noires, avec les tibias plus ou moins brunâtres; les fémurs médians n'ont pas un ctenidium typique.

Chétotaxie du thorax. ac = 0 + 1, dc = 4 – 5 + 4, ia = 1 + 2 (les présuturaux sont petits), prs = 1, sa = 3, pa = 2, h = 4, ph = 2, n = 4, sc = 3 + 1 (ap manquent, sap très longs), pp = 1 (plus 1 macrochète), pst = 1, st = 1:1:1.

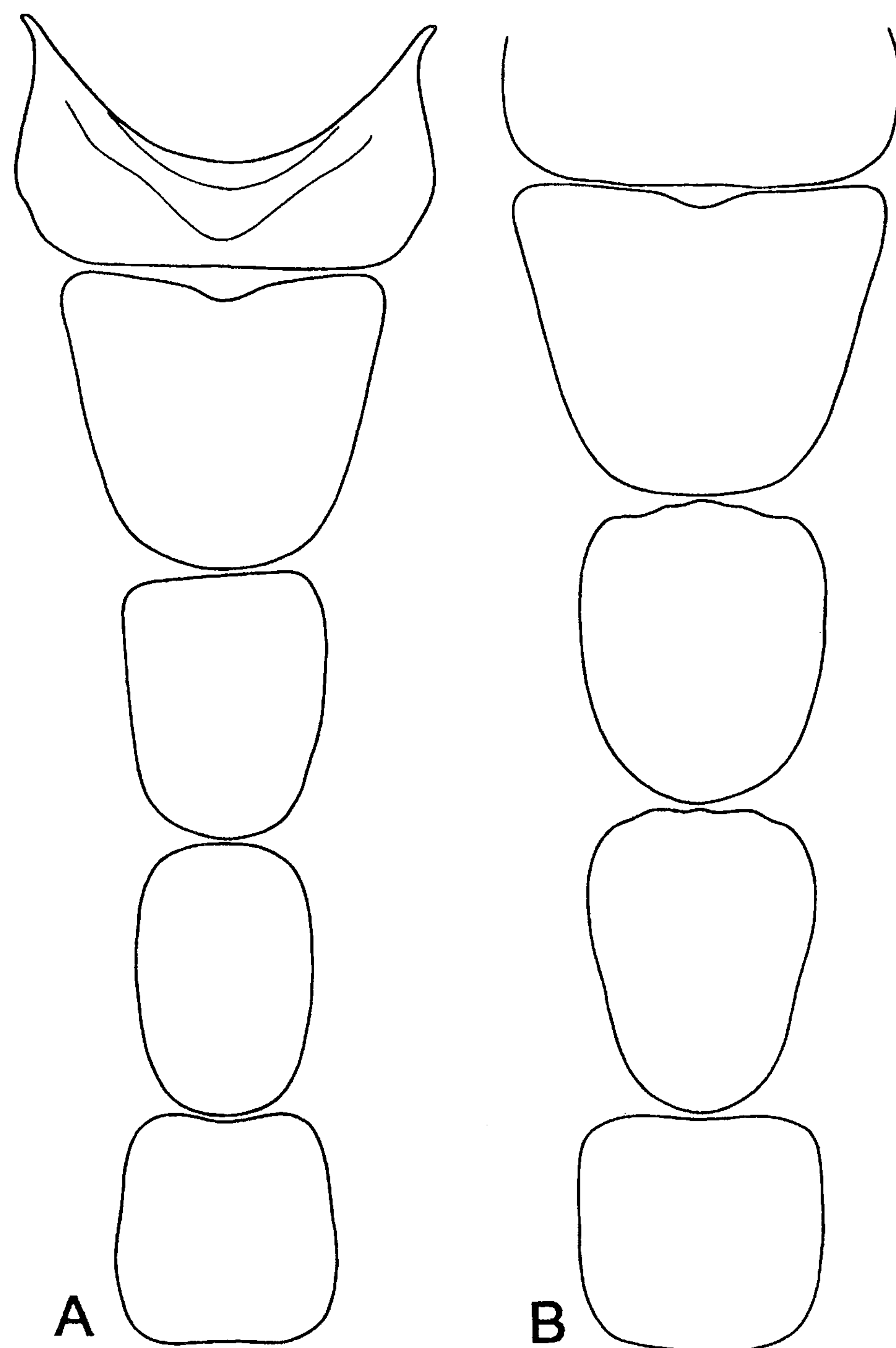

Fig. 2. — Sternites préabdominaux des femelles de *Kanoa okazakii* (Kano) (A) et de *Sarcotakaops arnaudiella* Lehrer (B).

Ailes. Transparentes. Epaulette est noire brunâtre; basicosta et costagium jaunes. La nervure r_1 est glabre. La nervure r_{4+5} est ciliée a peu près jusqu'à la proximité de la nervure r-m. Cubitulus est courbé en angle droit et prolongé d'un petit apendice-nervure et d'un pli. L'épine costale est de longueur moyenne. Les écailles sont blanches; les balanciers brunes noirâtre.

Chétotaxie des tibias. Les tibias antérieurs ont 3 ad proximaux assez développés et 1 pv. Les tibias médians sont pourvus de 2 ad, 1 av, 4–5 pd (desquels 2 pd grands) et 2 pv. Les tibias postérieurs ont 3 ad grands, 2 av et 2 pd.

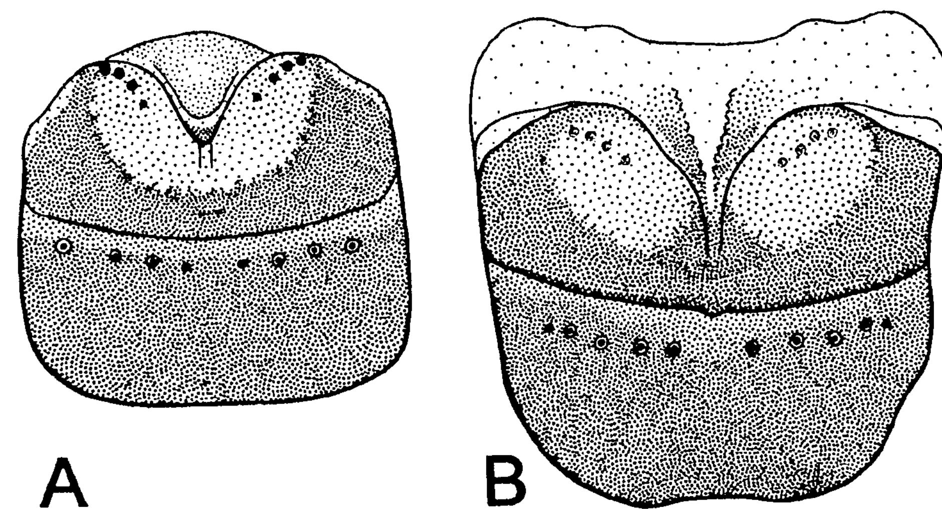

Fig. 3. — Sternites postabdominaux VI–VII des femelles de *Kanoa okazakii* (Kano) (A) et de *Sarcotakaops arnaudiella* Lehrer (B).

Abdomen. Noir, avec le tomentum argenté et les dessins en damiers. La formule chétotaxique: 0 + 0 + série + série. Tergite VI noir à tomentum argenté. Le sternite II (fig. 2) a une forme habituelle, étant le plus développée. Les sternites III et IV sont ovalaires, allongés et le premier a la marge antérieur droite. Le sternite V est rectangulaire, plus large que les précédents, avec les coins arrondis et un peu plus large dans la moitié postérieure. Le sternite VI (fig. 3) est plus ou moins rectangulaire et pourvu de 4 paires de macrochètes marginaux postérieurs forts. Le sternite VII est plus étroit et les deux hémisternites sont séparés par une profonde excavation ondulée, dans l'intérieur desquels se forme une zone bombée et un peu pigmentée.

Longueur du corps: 10 mm.

Matériel étudié. 1 femelle, avec l'étiquette: «Mt Takao, Tokio, 8 oct. 1961, col. R. Kano».

DIFFÉRENCES GÉNÉRIQUES ET SPÉCIFIQUES ENTRE LES FEMELLES DE *KANOA OKAZAKII* ET *SARCOTAKAOPS ARNAUDIELLA*

Celles-ci sont présentées dans les tableaux plus bas:

Kanoa okazakii (Kano, 1953):

- La bande frontale noire brunâtre jusqu'à noire est dépourvue des fins macrochètes croisées;
- Le troisième article de l'antenne est 2,5 fois plus long que le deuxième;
- les palpes sont longs, aplatis latéralement (non gonflés), en forme de raquette de tennis, avec un pédoncule long et quelques microchètes fins sur son bord antéro-superterminal;
- péristome mesure 1/4 du petit diamètre oculaire;
- les microchètes occipitaux sont disposés sur trois rangs irréguliers;
- ia =1 + 2; sc = 3 – 5 + 1;
- les tibias médians ont 2 ad, 1 av, 4–5 pd (desquels 2 pd sont grand) et 2 pv;
- le tergite VI de l'abdomen est noir à tomentum argenté;
- le sternite III est ovale, plus étroit et avec la marge antérieure droite;
- le sternite IV a les marges latérales parallèles et une forme allongée;
- le sternite V est rectangulaire, avec les coins arrondis, un peu plus long que large, étant creusé à la marge antérieure et ayant la moitié postérieure un peu plus large que celle antérieure;
- le sternite VI est plus ou moins rectangulaire, avec les coins antéro-latéraux arrondis, plus étroit, à marges subparallèles et pourvu de 4 paires de macrochètes marginaux postérieurs;
- le sternite VII a les hémisternites fortement ondulés à la ligne médiane et dans l'excavation d'entre eux se forme une zone bombée un peu pigmentée.

Sarcotakaops arnaudiella Lehrer 1985:

- la bande frontale noire a deux paires de macrochètes fins et croisés;
- le troisième article de l'antenne est plus petit que 2,5 fois de la longueur du deuxième;
- les palpes sont très développés, larges et aplatis latéralement, un peu courbés, à marges parallèles, un pédoncule très court, l'apex tronqué et avec quelques denticules sur son bord antéro-superterminal;
- le péristome mesure 1/3 du grand diamètre oculaire;
- les microchètes occipitaux sont disposés sur deux rangs;
- ia = 1 + 3(4); sc = 3 + 1;
- les tibias médians ont 2 ad, 1 av, 2 pd et 1 pv;
- le tergite VI de l'abdomen est noir luisant, partiellement d'un brun foncé et noir;

- le sternite III est ovale, mais avec la marge antérieure plus proéminente au milieu;
- le sternite IV est ovoïdale et plus large dans la moitié antérieur que dans celle postérieure;
- le sternite V est rectangulaire, à coins arrondis et un peu plus large que long;
- Le sternite VI est plus ou moins demi-circulaire, avec la partie postérieure plus large que celle antérieure, la marge antérieure un peu creusée au milieu et pourvu de 5 paires de macrochètes marginaux postérieurs;
- le sternite VII est un peu plus large dans sa partie antérieure, ses hémi-sternites sont plus ondulés et unis sur la ligne médiane par une zone plus pigmentée (mais nous ne connaisons exactement sa forme possible, parce qu'elle a été détruite un peu par les anthrènes).

Étant donné les nombreuses différences morphologiques entre ces deux femelles, la conclusion s'impose spontanément: *Sarcotakaops arnaudiella* Lehrer, 1985 et *Kanoa okazakii* (Kano, 1953) sont de bons et distincts espèces et genres de la faune paléarctique. Leur synonymie a été une regrettable erreur et la conséquence d'une analyse superficielle, qui n'a pas été fondée sur une recherche sérieuse du matériel de collection et n'aurait pas été possible seulement sur les données bibliographiques contradictoires de la littérature japonaise.

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BLACKITHIANA N. G., UN NOUVEAU GENRE PALÉARCTIQUE ET LA RÉDESCRIPTION DE SON ESPÈCE-TYPE *BLACKITHIANA ORNATIJUXTA* (RICHET et al., 1995) *(DIPTERA, SARCOPHAGIDAE)*

ANDY Z. LEHRER

A new genus (*Blackithiana* n. g.), on the basis of the structural type of the male genitalia of *Blackithiana ornatijuxta* (Richet, Pape, Blackith & Blackith, 1995), is established. The diagnosis of this species and the distinctive characters of the genus *Sarcophaga* Meigen, 1826 are present.

Récemment a été décrite la nouvelle espèce paléarctique *Sarcophaga ornatijuxta* par Richet, Pape, Blackith & Blackith (1955). Elle a été colligée tout d'abord dans le sud de la France (Pyrénées-Orientales, Amélie-les-Bains, octobre 1912, coll. Mus. Hist. Nat. Paris), retrouvée dans de divers départements (Aude, Hérault et Pyrénées-Orientales) et puis dans le nord-est de l'Espagne.

Après quelques considérations taxonomiques sur le plan de la structure du genre *«Sarcophaga sensu stricto»*, qui s'appuie sur une analyse superficielle et parfois inexacte de ses caractères, les auteurs arrivent à la conclusion que l'espèce *ornatijuxta* appartient à ce genre. Mais, vu que la représentation et l'interprétation des structures génitales spécifiques ne sont pas correspondantes et réelles, nous avons sollicité à nos distingués collègues Mme dr. Ruth et M. prof. Robert Blackith (Dublin) un exemplaire mâle pour vérifier les suppositions taxonomiques mentionnées et, éventuellement, pour compléter sa diagnose. Le résultat des recherches entreprises par nous a montré que son statut est totalement différent du genre *Sarcophaga Meigen,* elle constituant le type d'un nouveau genre (*Blackithiana* **n. g.**) pour la familie Sarcophagidae.

Il faut souligner que dans le travail de ces auteurs s'est écoulée une idée étrange concernant *«the narrow generic concepts currently employed by several authors»*, qui déterminent aujourd'hui que *«practically 50% of the genera of Sarcophagini are monotypic»* (Richet et al., 1995:431). D'après nous, les études complexes de taxonomie doivent refléter la délimitation correcte et la subordination réelle des unités fondamentales et non pas une statistique qui ne soit pas significative ou un groupement artificiel et chaotique de celles-ci, n'offrant qu'une apparente unité taxonomique. La diversité biomorphologique n'élimine pas la compréhension des relations phyllogénétiques, si on apprécie correctement tant les caractères courrants de morphologie externe, que ceux d'anatomie comparée du complexe génital.

REV. ROUM. BIOL.-BIOL. ANIM., TOME 42, N° 1, P. 11–17, BUCAREST, 1997

DIAGNOSE DU GENRE *BLACKITHIANA* n. g.

Synonymes:
= *Sarcophaga (s. str.)* sensu Richet, Pape, Blackith & Blackith (partim), 1995, Bull. Soc. Ent. France, **100** (4): 432 — **n. syn.**

Le front et la bande frontale sont très larges; le premier mesure plus de 2/3 de la largeur d'un œil et la dernière est 4 fois plus large qu'une parafrontalie. Le troisième article de l'antenne est un peu plus long que le double du deuxième. Le péristome mesure plus d'un 1/3 du grand diamètre oculaire. Propleures glabres. Les fémurs médians ont un cténidium typique et long. ac = 3 + 1; dc = 4 – – 5 + 4. L'abdomen a les dessins en damiers. Le tergite III est pourvu de 2 macrochètes médio-marginaux forts. Les tergites génital et anal sont noirs; le premier est pourvu de macrochètes marginaux. Le sternit V (fig. 1) n'a pas de brosses. Les cerques sont plus ou moins ondulés. La partie basale du paraphallus est triangulaire et prolongée des lobes paraphalliques cvadrangulaires. Juxta est énormément développée parallèlement sur l'axe du distiphallus, étant porvue de nombreuses épines de différentes dimensions.

Espèce-type: Sarcophaga ornatijuxta Richet, Pape, Blackith & Blackith, 1955.

DESCRIPTION DE *BLACKITHIANA ORNATIJUXTA* (RICHET et al., 1995)

MÂLE

Tête. Noire et couverte d'un tomentum argenté. Front large; vu du dessus et au niveau le plus étroit, il mesure 2/3 de la largeur d'un œil. La bande frontale noire est presque 4 fois plus large qu'une parafrontalie. Les antennes sont noires et plus luisantes sur les articles basaux; le troisième article est 2,25 fois plus long que le deuxième. Les parafaciales sont deux fois plus larges que la largeur du troisième article de l'antenne. La trompe et les palpes sont noirs, les derniers un peu élargis dans leur moitié apicale. Le péristome est un peu plus large que 1/3 du grand diamètre oculaire.

Chétotaxie de la tête. Les macrochètes verticaux internes sont rétroclines, mais moins longs et gros que d'habitude; les macrochètes verticaux externes piliformes à peine distincts; les ocellaires proclines sont petits et minces; les préverticaux rétroclines sont bien développés; les macrochètes frontaux sont au nombre de 11 paires; 4–5 macrochètes parafaciaux piliformes sont assez longs; les petites vibrisses montent jusqu'à la moitié des bordures faciales; on voit 2 postocellaires et 1 postvertical sur chaque partie de l'occiput; les microchètes

occipitaux sont disposés sur deux rangs. Le péristome est couvert de poils noirs et minces; la partie postérieure de la tête a de poils blancs.

Thorax. Noir, avec tomentum argenté, 3 bandes noires médio-dorsales longitudinales larges, 2 bandes paramédianes et 2 bandes latérales étroites. Propleures glabres; prosternum poilu. Les stigmates sont jaunes brunâtre. Les pattes sont noires, avec une faible teinte brune surtout sur les tibias postérieurs; les fémurs médians ont un cténidium typique et long; les fémurs médians et, surtout, les fémurs postérieurs ont un rang complet de macrochètes antéro-ventraux assez long, mais de la même longueur et semblable à un peigne rare.

Chétotaxie du thorax. ac = 3 + 1, dc = 4 – 5 + 4, ia = 1 + 3, prs = 1, sa = 3, pa = 2, h = 3, ph = 2, n = 4, sc = 4 + 1, pp = 1 (plus quelques macrochètes), pst = 1 (plus 1 macrochète), st = 1:1:1.

Ailes. Transparentes. Epaulette noire; basicosta et costagium sont jaunes. La nervure r_1 est glabre. La nervure r_{4+5} est ciliée sur un tiers de la distance entre son origine et la nervure transverse r-m. Cubitulus est courbé dans un angle un peu aigu et prolongé d'un pli. L'épine costale est petite. Les écailles sont blanches; les balanciers d'un jaune brunâtre.

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

Abdomen. Noir, avec un tomentum argenté et les dessins en damiers. La formule chétotaxique: 0 + 2 + 2 + série. Le tergite génital est long, noir luisant et porvu de 4 paires de macrochètes forts. Le tergite anal est noir.

Armature génitale: fig. 1–3. Le sternite V (fig. 1) n'a pas de brosses; il est assez grand, avec la base longue mais plus étroite et présente un foramen triangulaire grand; les lames latérales sont plus étroites qu'au genre Sarcophaga Meigen, avec les sommets arrondis; les condyles sont plus ou moins ovales et relativement petits. Les cerques (fig. 3, A) ont une forme différente que celle des espèces du genre *Sarcophaga* et, surtout, de *S. marcelleclercqi* Lehrer; ils sont plus larges dans leur zone subapicale, largement courbés à la marge dorsale, avec la marge ventrale ondulée, les sommets aigus et éloignés vers l'extérieur; les paralobes sont plus ou moins triangulaires et relativement larges. Theca est un peu plus longue que le distiphallus, ayant développé seulement la paroi dorsale et la partie proximale. Distiphallus (fig. 2) est moyen et le paraphallus n'est pas séparé en parties mobiles articulées. La partie basale du paraphallus est haute, triangulaire et se prolonge dorsalement avec une paroi très large et dans la région antéro-inférieure avec les lobes paraphalliques assez grands et plus ou moins cvandrangulaires. Les styles sont orientés en avant et presque perpendiculairement sur l'axe longitudinal du distiphallus; ils sont longs, larges, tubulaires et dépourvus de dents récurrentes. La partie apicale du

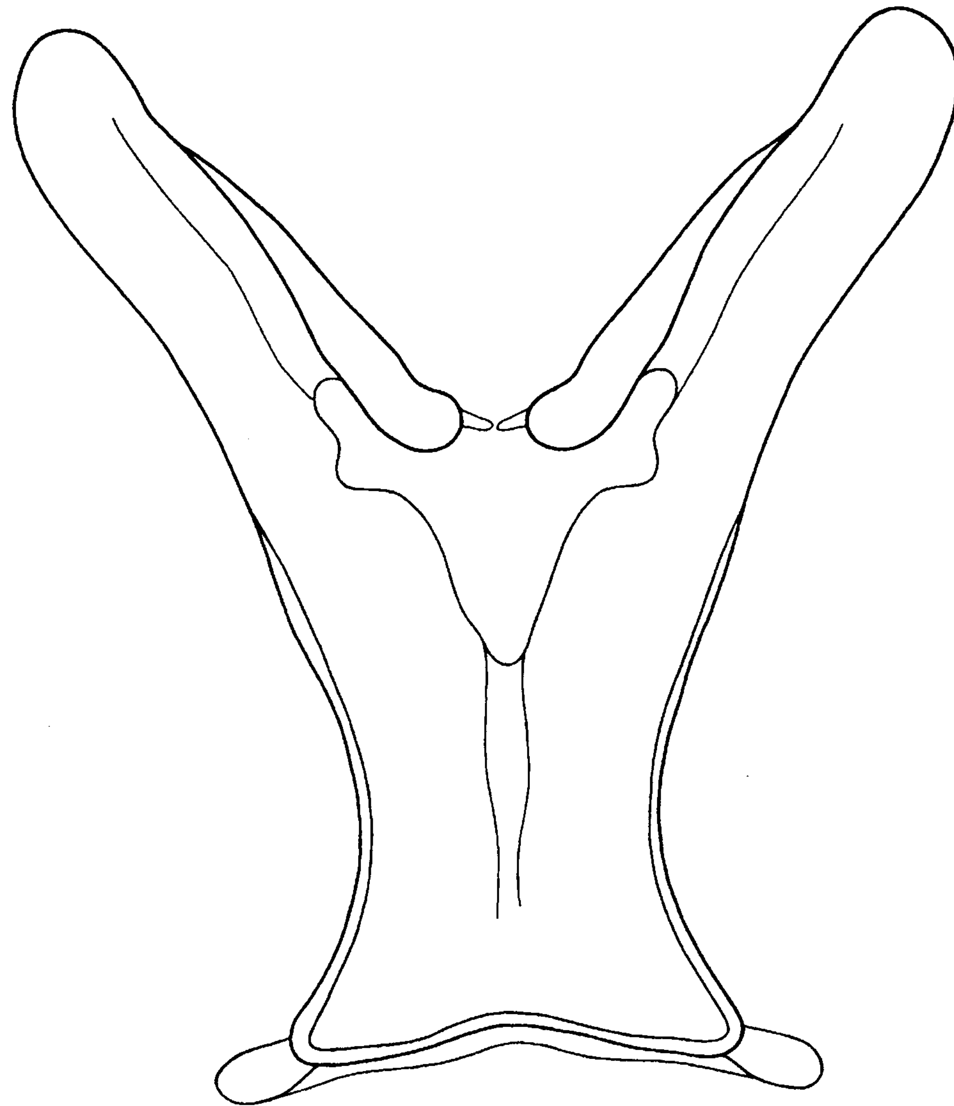

Fig. 1. — *Blackithiana ornatijuxta* (R.P.B.B.); sternite V.

paraphallus est formée seulement d'une juxta extraordinairement développée perpendiculairement, membraneuse, un peu pigmentée, dépassant beaucoup la partie dorsale du paraphallus et pourvue de nombreuses épines de différentes longueurs. Les lobes hypophalliques ont une base très sclérifiée et pigmentée,

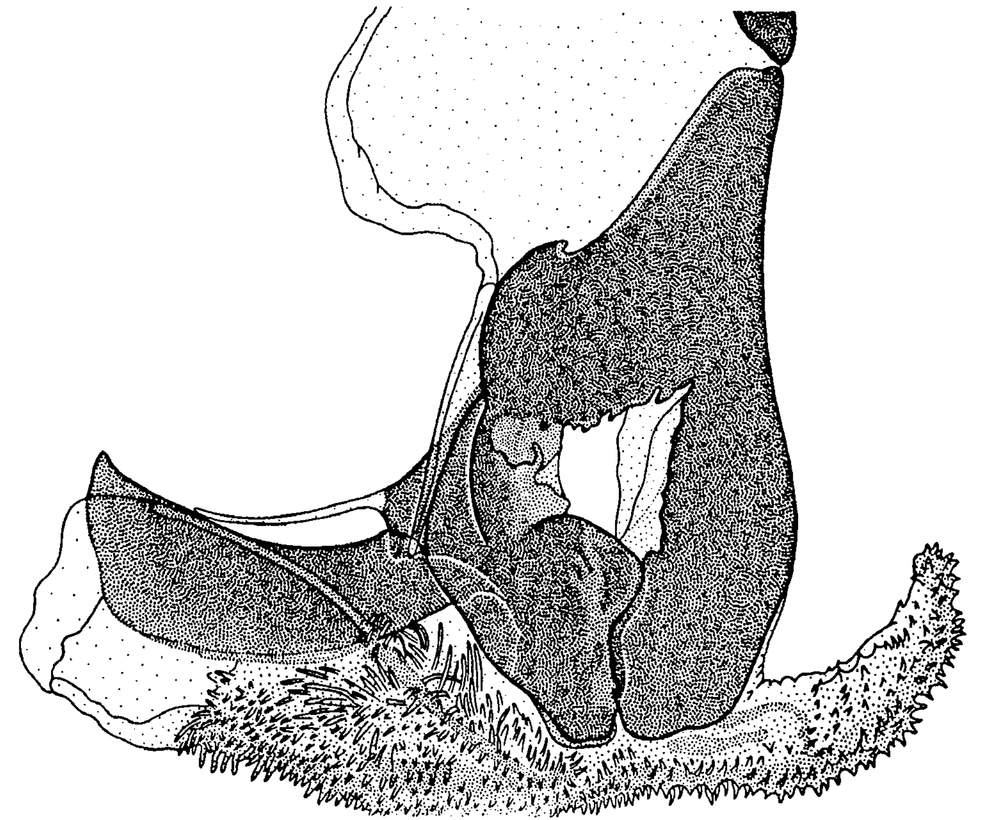

Fig. 2. — *Blackithiana ornatijuxta* (R.P.B.B.); distiphallus.

en se prolongeant jusqu'au tiers terminal des styles sous une forme filiforme. Membrana est très grande et a une paire de lobes membranaux très longs, minces, sous la forme de stylets transparents. Les prégonites (fig. 3, B) sont plus longs que les postgonites (fig. 3, C); les premiers sont courbés et avec les sommets aigus; les seconds ont la base plus large, le sommet émoussé et deux macrochètes petits superterminaux.

Longueur du corps: 13 mm.

FEMELLE. Inconnue.

Matériel étudié. 1 mâle avec les données suivantes écrites par la main du Prof. Blackith: «France, Hérault, Gorge de l'Hérault (linestone). 19 May 1955. Sarcophaga ornatijuxta Richet et al. Coll. et det. B. & B. Blackith 1995».

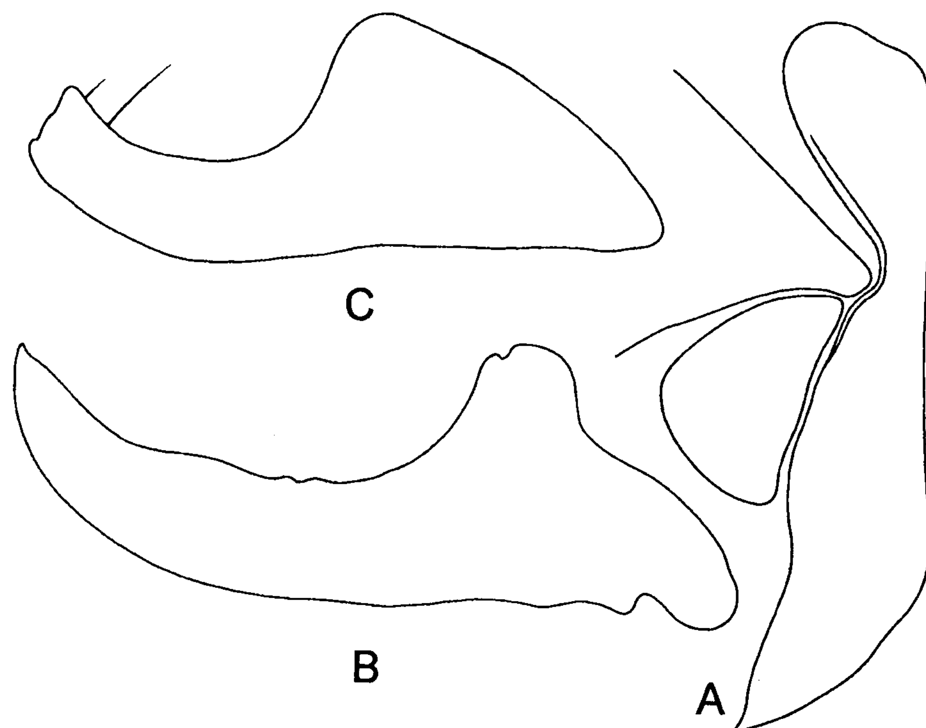

Fig. 3. — *Blackithiana ornatijuxta* (R.P.B.B.); A: cerques et paralobes; B: prégonites; C: post-gonites.

COMMENTAIRES

Pour éliminer toute confusion concernant le statut de *Blackithiana ornatijuxta* (R.P.B.B.) il est nécessaire de réconsidérer la diagnose du genre *Sarcophaga* Meigen, 1826. Ce genre est caractérisé par les trait suivants:

Le front est peu proéminent et mesure, vu du dessus et au lieu la plus étroite, entre 1/2–2/3 de la largeur d'un œil. Les parafaciales sont plus étroites que le double de la largeur du troisième article de l'antenne. Le péristome mesure entre 1/4–1/3 du grand diamètre oculaire (excepté le sous-genre *Fernandamyia* Lehrer 1975, où il mesure presque 1/2 du grand diamètre oculaire). ac présuturaux manquent; dc postsuturaux sont au nombre de 4–5. Les propleures sont glabres. Les fémurs médians ont un cténidium typique bien développé. Les tergite génital et anal sont noirs, le premier ayant quelques paires de macrochètes marginaux (excepté le sous-genre *Fernandamyia,* où les segments postabdominaux sont bruns-rougeâtre et le tergite génital dépourvu de macrochètes marginaux). Sternite V n'a pas de brosses, mais les lames latérales sont longues, avec les marges parallèles et les sommets arrondis. Les cerques sont légèrement cour-

bés ventralement (excepté le sous-genre *Fernandamyia*), avec les branches rapprochées, subparallèles et sans être pliés dorsalement dans leurs tiers proximal. Distiphallus court. La partie basale du paraphallus est étroite; la partie apicale du paraphallus est fine, souvent rudimentaire comme la juxta membraneuse. Les styles sont droits, massifs et gros (excepté le sous-genre *Fernandamyia*), parfois courbés au sommet et pourvus toujours de dents récurrentes. Les lobes hypophalliques sont relativement gros et longs. Les prégonites, d'habitude, sont longs, élargis et avec une crête medio-longitudinale perpendiculaire; les postgonites sont plus ou moins en forme de crochets. Les lobes membranaux sont situés distalement par rapport aux lobes paraphalliques ventraux.

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THE STATUS OF SOME NOMINAL GENERA OF EURASIAN CYPRINIDAE (OSTEICHTHYES, CYPRINIFORMES)

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The author lists a nominal genus of Eurasian cyprinids overlooked by Eschmeyer in 1989, (*Pseudobarbus* Bielz, 1853, not of Smith, 1849, actually a synonym of *Barbus*), four genera described after 1949 (all considered valid), discusses the status of 11 genera, mentions the papers in which 60 nominal genera have been put for the first time in synonymy and those in which 15 other genera have been validated (or resurrected from synonymy), mentions the true synonymy of eight nominal genera for which Eschmeyer has given a wrong synonymy and considers valid 15 nominal genera (some of these being validated only on the base of their descriptions). The status of several nominal genera from Eurasia remains doubtful (unclarified).

Eschmeyer's (41) list of the nominal genera of recent fishes described until the end of 1989 replaces the since long time outdated "Genera of Fishes" (47). The list mentions, for many genera, their present status (valid or synonym), according to one or several recent contributions, contradictory opinions being often mentioned.

The aim of the present contribution is to list a few nominal genera of Eurasian Cyprinidae, overlooked by Eschmeyer or described after January 1990, to clarify the status of some genera and to mention, when possible, the first author and paper in which a genus has been synonymized or validated. The type-species of the genera listed by Eschmeyer are mentioned only when synonyms.

LIST OF THE GENERA

Aaptosyax Rainboth, 1991; type: *A. grypus* Rainboth. Valid (according to the description) (65).

Acanthobrama Heckel, 1843; its validation (Yang in 80) mentioned by Eschmeyer cannot be accepted, since it refers to a species (*A. simoni*) that actually belongs to *Pseudobrama* Bleeker (37, 5). First right validation: Goren et al. (42).

Acanthorutilus Berg, 1912. Synonym of *Oreoleuciscus* (32). The Anatolian species included by some authors in *Acanthorutilus* actually belong to *Pseudophoxinus* (33).

Alburnoides Jeitteles, 1861; type *Alburnus maculatus* Kessler 1859 = *Cyprinus bipunctatus* Bloch, 1782. Unanimously accepted as valid.

Adamacypris Fowler, 1934; first synonymized with *Puntioplites* by Smith (74).

REV. ROUM. BIOL.–BIOL. ANIM., TOME 42, N° 1, P. 19–30, BUCAREST, 1997

Altigena Lin, 1933; first synonymized with *Sinilabeo* by Bănărescu (17).

Anabarilius Cockerell, 1923. Considered a synonym of *Hemiculterella* by Bănărescu (9); actually valid (81,45, also Bănărescu, unpublished).

Ancherythroculter Yih & Wu, 1963. Valid (Bănărescu) (9).

Aphyocyprioides Tang, 1942. Status unclarified, neither the genus nor the type species being mentioned, as valid or synonym in recent bibliography.

Armatogobio Taranets, 1937. First synonymized with *Saurogobio* by Berg (27), eventually valid subgenus; comprising five of the six species of the genus (20).

Aspiobarbus Berg, 1932. Original description (28) not known to Eschmeyer: Zoogeographica, 1: 148; type *Barbus comiza* Steindachner by original designation. Synonym of *Barbus*: synonymized by Almaca (2).

Bangana Hamilton, 1822: type *Cyprinus (Bangana) dero* Hamilton. Synonymized with *Labeo* by Day (39). The type species is listed as *Labeo dero* in the recent comprehensive works on the fishes of India (46, 76). However, Rainboth (66) mentions *Bangana* as valid genus, comprising species in east and south Asia.

Barbodon Dybowsky, 1872. First synonymized with *Sarcocheilichthys* by Berg (27).

Bathystoma Fitzinger, 1873. First synonymized with *Leuciscus* by Berg (26).

Bengala Gray, 1873 (only illustration, not description). Validated by Talwar and Jhingran (76); replaces *Megarasbora* Gunter with the same type species, *Cyprinus elanga* Hamilton. Eschmeyer (41) suggests that *Bengala* in Gray's plate 96 is a mispelling for *Bengana*, type *Cyprinus (Bengana) falcata*, illustrated on plate 97. Actually the two specific names and illustrations are different.

Bengana Gray, 1832 (illustration only). Status obscure, the type species being not identified.

Boraras Kottelat & Vidthayanon, 1993; type *B. micros* Kottelat & Vidthayanon. Valid (according to the description) (55).

Brachygramma Day, 1865; type *B. jerdonii* Gray, 1865 = *Cyprinus gotyla* Gray, 1832. Synonym of Garra, the type species, *C. gotyla* being now placed in *Garra* (59).

Brevigobio Tanaka, 1916; probably synonym of *Hemmigrammocypris* (29).

Bungia Keyserling, 1861. First synonymized with *Gobio* by Berg (27).

Bliccopsis Heckel, 1843. Invalid, based on an intergeneric hybrid.

Cachius Gunther, 1868. First synonymized with *Chela* by Silas (72).

Candidia Jordan & Richardson, 1909; synonym of *Zacco* according to Bănărăscu (12), of *Opsariichthys* according to Howes, valid according to Chen (35).

Carasobarbus Karaman, 1971. Valid (personal opinion).

Caraspius Nichols, 1925. Synonymized with *Aphiocypris* by Nichols in 1943 (63).

Carinozacco Zhu et al; 1982. In my opinion a synonym or subgenus of *Zacco*.

Carpio Heckel, 1843. Invalid, based on an intergeneric hybrid.

Cephalacompsus Herre, 1924. Probably a synonym of *Puntius*, like all presumed cyprinid genera endemic to Lake Lanao, Philippines (53).

Cephalopsis Fitziger, 1873. Synonym of *Leuciscus*, subgenus *Squalius* (26).

Cephalus Bonaparte, 1846. Synonym of *Leuciscus*, subgenus *Squalius* (26).

Chanodichthys Bleeker, 1859; type *Leptocephalus mongolicus* Basilewski, 1855, which is the same as *Culter mongolicus* Basilewski, 1855, as understood by most recent authors and placed in *Erythroculter*, as *Erythroculter mongolicus*. *Chanodichthys* is hence a senior synonym of *Erythroculter* Berg, 1909; it is preferable however to retain the latter as nomen conservandum, being in general use and comprising commercially valuable species and to declare *Chanodichthys* as a nomen oblitum.

Chela Hamilton, 1822. Validated in its present acceptance by Silas (72).

Chilogobio Berg, 1914. Synonymized with *Sarcocheilichthys* first by Mori (60).

Coripareius Garman, 1912; type *Labeo cetopsis* Kner = *Gobio heterodon* Bleker. First synonymized with *Coreius* by Tchang (78).

Culticula Abbott, 1901; type *C. emmelas* Abbott = *Acanthobrama simoni* Bleeker. First synonymized with *Pseudobrama* by Chu (37).

Cultrichthys Smith, 1938; type *Culter brevicauda* Gunther = *C. alburnus* Basilewski. Synonym of *Culter* Basilewski which is placed in the Official List of generical names (67).

Cutrops Smith, 1938. First synonymized with *Paralaubuca* by Bănărescu (9).

Erythroculter Berg, 1909; type *Culter erithropterus* Basilewski; it is questionable however whether the species identified by Berg as *erythropterus* is the same as that described by Basilewski under this name; it is surely identical with *Culter ilishaeformis* Bleeker (80). *Erythroculter* is a junior synonym of *Chanodichthys* (37); it is preferable however to retain it as nomen conservandum and to reject *Chanodichthys* as nomen oblitum.

Exoglossops Fowler & Been, 1920; type *E. geei* = *Sarcocheilichthys sinensis* Bleeker, 1871. First synonymized a with *Sarcocheilichthys* by Rendahl (67).

Danionella Roberts, 1886. Valid (according to description, 69).

Discherodontus Rainboth, 1989. Valid (according to description).

Folifer Wu, 1977, as subgenus of *Tor*. In my opinion it deserves specific rank.

Fundulichthys Bleeker, 1859; type *Fundulus virescens* Temminck & Schegel = *Leuciscus parvus* Temminck & Schegel. First synonymized with *Pseudorasbora* by Berg (27).

Fusania Jordan & Starks, 1905; type *F. ensarca* J. & S. = *Aphiocypris chinensis* (according to Nichols, 63). Synonym of *Aphiocypris.*

Gobiobarbus Dybowski, 1869. First synonymized with *Hemibarbus* by Berg (27).

Gobiocypris Ye & Fu, 1983. Valid according to description and illustration (82).

Gobiosoma Dybowski, 1872. First synonymized with *Saurogobio* by Berg (27).

Gonoproktopterus Bleeker, 1860 as subgenus of *Hypselobarbus.* Valid genus, comprising six species in southwestern India (76). The Korean *Barbus mylodon* Berg, formerly ascribed to this genus, actually belongs to *Hemibarbus* (24).

Gymnodanio Chen & He, 1992, type: *G. strigatus* Chen & He. Valid (according to description, 36).

Gymnognathus Sauvage, 1884; first synonymized with *Elopichthys* by Chu (37).

Gymnostomus Heckel, 1843; type *Cyprinus ariza* Hamilton. Eschmeyer follows Leveque & Daget (57) considering this genus a synonym of the exclusively African *Varicorhinus* (46, 76). Actually it is a synonym of *Labeo*, to which the type species belong. The western Asian species formerly in *Gymnostomus* belongs to *Capoeta* (50) the East Asian ones to *Onychostoma* (14).

Heteroleuciscus (intendend: *Heteroleuciscus*) Sauvage, 1874; synonym of *Hampala* (Kottelat, according to personal information from Dr. M. L. Bauchot).

Holotylognathus Fowler, 1934; type *H. reticulatus* Fowler, 1934 = *Crossocheilus reticulatus* Fowler, 1935, the species having been described by the same author twice, with the same specific name but ascribed to distinct genera. Synonymized with *Crossocheilus* independently by Bohlke (34) Bănărescu (19).

Horalabiosa Silas, 1954. Status unclarified, the genus and the type species being not mentioned in recent works (46,76) as either valid of synonym being however considered valid by Menon (manuscript paper).

Huigobio Fang, 1938. First synonymized with *Microphisogobio* by Bănărescu and Nalbant (23, 24) recently accepted as eventual valid subgenus (20).

Idus Heckel, 1843. Unanimously accepted as synonym or subgenus of *Leuciscus*.

Kendallia Evermann & Shaw, 1927. First synonymized with *Hemiculter* by Chu (37).

Kosswigobarbus Karaman, 1971. Valid (personal opinion).

Ladigesocypris Karaman, 1972; valid (40).

Ladislavia Dybowski, 1869. First validated by Berg (27).

Laichowcypris Hao & Hoa (year?), type *Laichowcypris dai* Hao & Hoa. A genus and species listed by Yen in 1985 (84), but not in an earlier publication of

the same author (83), without any indication of the paper in which these have been described.

Laubuca Bleeker, 1859. Apparently first synonymized with *Chela* by Smith (74).

Leptocephalus Basilewski, 1855; preoccupied. Senior synonym of *Chanodichthys* Bleeker, 1859 or of *Erythroculter* Berg, 1909, if this is accepted as nomen conservandum.

Leucos Heckel, 1843; type *L. cisalpinus.* Status unclarified, the position of the type species being not established. Its name suggests occurrence in northern Italy, hence the species may by a synonym of the North Italian *Rutilus aula* (not to the South Italian *R. rubilio,* 31) and *Leucos* a synonym of *Rutilus* s.str.

Lissochilus Weber & Beaufort, 1916; preoccupied. Most authors including Eschmeyer (41) consider *Lissochilus* a senior synonym of *Acrosscheilus* Oshima. Actually both genera are distinct, differing mainly in the shape of the mouth *Acrossocheilus* is exclusively East Asian; the southern Asian species formerly in *Lissochilus* (including *L. sumatranus*, the type species) are presently assigned to *Neolissochilus* Rainboth, 1985, *Lissochilus* being its senior synonym; a few may belong to *Poropuntius.*

Longurio Jordan & Stark, 1905; type: *L. athymius* = *Saurogobio dumerili.* First synonymized with *Saurogobio* by Berg (27).

Machaerochilus Fitzinger, 1873. It is not clear who was the first to synonymize it with *Chondrostoma.*

Mandibularca Herre, 1924. Probably synonym of *Puntius,* like all presumed endemic genera from Lake Lanao, Philippines (53).

Masticbarbus Tang, 1942. Status not clear, the type species having never been found again. Its illustration (77) suggests similarity with *Acrossocheilus.*

Mayoa Day, 1870; type: *M. modesta* Day = *Platycara lissorhynchus.* First synonymized with *Discognathus* (i. e. with *Garra*) by Day (39).

Mearnsella Sean & Bean, 1907. First synonymized with *Nematabramis* by Berg (25).

Megagobio Kessler, 1876. First synonymized with *Rhinogobio* by Rendahl (68).

Mesopotamichthys Karaman, 1971. Valid (personal opinion).

Metzia Jordan & Thompson, 1914; synonymized with *Rasborinus* by Berg (29).

Micraspius Dybowski, 1869; type: *M. mianowskii* = *Leuciscus parvus* Temminck & Schegel. First synonymized with *Pseudorasbora* by Berg (27).

Microphysogobio Mori, 1934. This valid genus has long been considered as exclusively Korean. Bănărescu & Nalbant (22, 23, 24) ascribed to it many species from China, the Amur basin and northen Vietnam, formerly in *Pseudo-*

gobio, *Rostrogobio* and *Huigobio,* some of which have latter been wrongly ascribed to *Abbottina* (Lo et al. in 81).

Morara Bleeker, 1859. First synonymized with *Aspidoparia* by Day (39).

Myloleucops Cockerell, 1913 and *Myloleucus* Gunther 1873, with the same type species, *Leuciscus aethiops* Basilewski = *L. piceus* Richardson are objective synonyms of *Mylopharyngodon* Peters, 1880, not of *Leuciscus,* as asserted by Eschmeyer (41).

Myloleucus Cope, 1872 (not of Gunther, 1873) is synonym of either *Mylocheilus* Agassiz, 1855 or *Mylopharodon* Ayres 1855, both native in western North America, not of the East Asian *Mylopharyngodon* as asserted by Eschmeyer (41).

Myloleuciscus Garmar, 1912, type *M. atripinnis* Garmar = *Leuciscus piceus* Richardson. First synonymized with *Mylopharyngodon* by Berg (26).

Nandina Gray, 1831. First synonymized with *Labeo* by Day (39).

Neochela Silas, 1858, as subgenus. Accepted as valid subgenus (11).

Neolissochilus Rainboth, 1985. Valid (54); replaces *Lissochilus,* preoccupied.

Nukta Hora, 1942, as subgenus. Accepted as valid subgenus of *Schismatorhynchus* by Jayaram (46).

Nuria Valenciennes, 1842. First synonymized with *Esomus* by Ahl (1).

Onychostoma Gunther, 1896. Valid (14) wrongly included by some authors in either *Varicorhinus* or *Gymnostomus.*

Oreoleuciscus Warpachowski, 1897. Apparently first validated by Berg (26).

Orfus Fitzinger, 1873. First synonymized with *Rutilus* by Berg (26).

Orthroleucus Derzhavin, 1937, as subgenus. Apparently valid subgenus of *Rutilus,* including the species from Transcaucazia, Anatolia, the western Balkan southern Italy and possibly Greece.

Ospatulus Herre, 1924; probably synonym of *Puntius,* like the other presumed endemic genera of cyprinids from lake Lanao (53).

Owsianka Dybowski, 1862. First synonymized with *Leucaspius* by Berg (26).

Pachychilon Steindachner, 1882. Valid (75), wrongly synonymized earlier with *Rutilus.*

Paracheilognathus Bleeker,1863. Considered synonym of *Acheilognathus* by Arai & Akai (3), but valid according to most other authors.

Parapelecus Gunther, 1889; type *P. argenteus* Gunther = *Pseudolaubuca sinensis* Bleeker. First synonymized with *Pseudolaubuca* by Bănărescu (6).

Paraphoxinus Bleeker, 1863; type species actually *Phoxinellus alepidotus* as demonstrated by Trewavas (79). Synonym of *Phoxinellus* with the same type-species.

Parapsilorhynchus Hora, 1921. Considered a synonym of *Garra* by Roberts (70), an opinion hardly acceptable. Jarayam (46) considers *Parapsilorhynchus* valid, Talwar & Jhingram even as representing a distinct family.

Pararasbora Regan, 1908. Status doubtful; possibly invalid.

Pararutilus Berg, 1908. Valid subgenus of *Rutilus* (26).

Paraschizothorax Bleeker, 1843. Synonym of *Schizopyge* Heckel, 1847, to which the type species *Schizothorax huegelii,* is presently ascribed (46) Talwar & Jhingram (76) include this species and its congeners in *Schizothoraichthys* Misra, 1962, which is a junior synonym of both *Schizopyge* and *Paraschizothorax* Bleeker.

Paraschizothorax Tsao, 1964; type: *Schizotorax* o'conneri Lloyd. Preoccupied *Tetrastichodon* Tchang, Yeuh & Hwang, with the same type species can be considered a replacement name, although not mentioned as such. The type species is ascribed, in recent papers, to *Schizothorax,* hence *Tetrastichodon* actually is as synonym of the latter.

Parasinilabeo Wu, 1939. Synonymized with *Crossocheilus* by Bănărescu (19).

Parator Wu & al., 1963; type: *Tor zonatus.* Considered a subgenus of *Tor* by Chu & Chen (38); it seems more closely related to *Spinibarbus* (16).

Parazacco Chen, 1982. Objective synonym of *Carinozacco,* which must be considered a subgenus of *Zacco.*

Parosteobrama Tchang, 1930. First synonymized with *Megalobrama* by Berg (30).

Phoxinellus Heckel, 1843; as demonstraded by Trewavas (79) the type species actually is *Phoxinellus alepidotus,* not *P. zeregi,* as considered by earlier authors and by Eschmeyer (41). In this acceptance, *Phoxinellus* replaces *Paraphoxinus* and includes the group of interrelated western Balkan species, the southern Balkan, Anatolian and Near East formerly in *Phoxinellus* being assigned to *Pseudophoxinus;* Karaman (51) includes also the latter group in *Phoxinellus,* synonymizing *Pseudophoxinus* with it.

Phoxiscus Oshima, 1919; type: *P. kikuchii* Oshima. Synonym of *Aphyocypris* to which the species *kikuchii* is presently ascribed (63).

Platycara Mc Clelland, 1839. Synonymized by Day (39) with *Discognathus* although *Platycara* has priority. Both nominal genera are synonym of *Garra, Platycara* being formally listed as synonym of the latter (39).

Platysmacheilus Lu. Luo & Chen, 1977. Synonym or subgenus of *Microphysogobio* (20).

Pseudobarbus Bielz, 1853; type *P. leonhardi* Bielz = *Barrbus petenyi* Heckel, conspecific with *B. peloponnesius* Valenciennes. A nominal genus, overlooked both by Jordan (47) and by Eschmeyer (41), although listed in books

of wide use (7,30). Preoccupied by *Pseudobarbus* Smith, 1841. Synonym of *Barbus*.

Pseudobrama Bleeker, 1870; type *P. dumerili* Bleeker 1870 = *Acanthobrama simoni* Bleeker, 1860. Validated by Chu (37) and Bănărescu (5). The single species of genus, *P. simoni* (Bleeker), restricted to China, is however still listed, in recent Chinese publications, as member of the western Asian *Achantobrama*.

Pseudoculter Bleeker, 1859; type *Culter pekinensis* Basilewski. Status uncertain. Lin (58) suggests that *C. pekinensis* may be the same as *Leptocephalus mongolicus* Basilewski, type species of *Chanodichthys* Bleeker. In this case, *Pseudoculter* becomes a valid genus, replacing both *Chanodichthys* Bleeker and *Erythroculter* Berg.

Pseudohemiculter Nichols & Pope, 1927. Considered synonym of *Hemiculter* by Bănărescu (9), valid genus by Chen (80). Perhaps valid subgenus.

Pseudolaubuca Bleeker, 1865. Validated by Bănărescu (6), later by Chu & Chen (38). Replaces *Parapelecus* Gunther.

Pseudoperilampus Bleeker, 1863. Synonymized with *Rhodeus* by Chen & Li (80), with *Acheliognathus* by Arai & Akai (3); actually valid (Holcik, in lit.).

Pseudophoxinus Bleeker, 1859; type *Phoxinellus zeregi* Heckel (79). Valid; replaces *Phoxinellus* as used until 1971. Considered a synonym of *Phoxinellus* by Karaman (51) but as distinct by Bogutskaya (33); the latter opinion is adopted here.

Pseudoxygaster Bănărescu, 1967; synonymized with *Seccuricula* by Howes (43).

Pteropsarion Gunther, 1868. Synonymized with *Barilius* first by Day (39).

Puntioplites Smith, 1929. Valid.

Rasborella Fowler & Bean, 1934. Status uncertain, not listed recently.

Rhinogobiodes Rendahl, 1928, as subgenus of *Gobio*. Synonymized with *Rhinogobio* by Rendahl in 1932 (68).

Rostrogobio Taranetz, 1937. Synonymized with *Microphysogobio* first by Bănărescu & Nalbant in 1965 (22). Considered valid genus by Lu, Luo & Chen (in 81); can eventually be accepted as subgens of *Microphysogobio* (20).

Salmophasia Swainson, 1939. Synonym of *Salmostoma*, with the same type species which has page priority.

Salmostoma Swainson, 1939.Valided (resurected from synonymy) by Bănărescu (10).

Scaphestes Oshima, 1919. Subgenus of *Onychostoma* (14,15) not of *Varicorhinus*, as assumed by Wu et al. (in 81).

Scaphiodonichthys Vinciguerra, 1890. Valid (74, 18), not synonym of *Cyprinion* as assumed by Howes (44).

Scaphiodontopsis Fowler, 1934. Synonym of *Scaphiodonichthys* (74, 18) not of *Cyprinion* as believed by Howes (44).

Securicula Gunther, 1868, as subgenus of *Chela*. First validated as genus by Howes (43). Replaces *Pseudoxygaster* with same type species.

Semiculter Chu, 1935. Synonymized with *Hemiculterella* by Bănărescu (9).

Sinibarbus Sauvage, 1874; type *S. viztatus* Sauvage 1874. Status obscure: the type and only known specimen is lost (37), the species has never been found again. Its short description (D III 8 with the last unbranched ray spinified and serrate, L I.40, phar. t. on one row, mouth subterminal, one pair of barbels) suggest that it is distinct from all known East Asian genera of the family.

Sinibrama Wu, 1939. Valid genus according to Yih & Wu (in 80), subgenus of *Megalobrama* according to Bănărescu (9).

Sinigobio Chu, 1935. First synonymized with *Squalidus* by Bănărescu (4).

Siniichthys Bănărescu, 1970. Never found again; possibly an intergeneric hybrid.

Sinilabeo Rendahl, 1932. Considered valid subgenus of *Varicorhinus* by Nichols (63), validated as genus by Bănărescu (17), an opinion endorsed by Wu & al. (in 81) and by Chu & Chen (38).

Similiogaster Bleeker, 1859. Listed by Eschmeyer (41) as a synonym of *Rohtee*, without references. The type species, *Leuciscus belangeri* Valenciennes is however ascribed to *Osteobrama* (46,76); *Smiliogaster* is hence a synonym of the latter.

Spinibarbichthys Oshima, 1926. Synonymized with *Spinibarbus* first by Bănărescu (16), latter by Chu & Chen (38).

Spinophoxinellus Karaman, 1972, as subgenus of *Phoxinellus*. Synonym of *Pseudophoxinus*, since Bogutskaya (33) lists the type species, *Phoxinellus anatolicus*, as species of *Pseudophoxinus*, without mentioning *Spinophoxinellus* as valid subgenus.

Spratellicypris Herre & Myers, 1931. Probably synonym of *Puntius* (53).

Squalalburnus Berg, 1932. Synonymized with *Alburnoides* by Berg (30). Possibly a valid subgenus of the latter.

Squalidus Dybowsky, 1872. Validated as subgenus of *Gobio* (4), later as valid genus (21); replaces *Sinigobio* Chu.

Squalius Bonaparte, 1837. Unanimously accepted as subgenus or synonym of *Leuciscus*.

Systomus Mc Clelland, 1939. Listed by Eschmeyer as synonym of *Barbus* actually synonym of *Puntius*, since the type species *S. immaculatus* Mc Clelland is a synonym of *Barbus sarana* (39), now in *Puntius* (46, 76).

Tambra Bleeker, 1860 (as subgenus of *Hypselobarbus*). Synonym of *Catla* Valenciennes, its type species, *Cyprinus abramoides* Sykes, being a synonym of *Cyprinus catla* Hamilton (39).

Telestes Bonaparte, 1833. Almost unanimously recognizet as valid subgenus of *Leuciscus*.

Tetrastichodon Tchang, Yueh & Hwang, 1964; type *Schizothorax o'conneri*. Same as *Paraschizothorax* Tsao, 1964, preoccupied. Probably a synonym of *Schizothorax* (see remarks on *Paraschizothorax*).

Tropidophoxinellus Stephanidis, 1974. Valid (40), but probably includes only one (*spartiaticus*) of the two Greek species ascribed to it and surely not the Iberian *"Phoxinellus"* or *"Tropidophoxinellus" alburnoides* (Steindachner).

Tylognathoides Tortonese, 1937. Probably a synonym of *Hemigrammocapoeta*.

Tylognathus Heckel, 1843, type: *Varicorhinus diplostomus* Haeckel. Eschmeyer (41) follows Karaman (50) in accepting this nominal genus as synonym of *Crossocheilus*; actually it us a synonym of *Labeo*, to which the type species, *V. diplostomus* belongs (46,76). The western Asian species often ascribed to *Tylognathus* actually belongs to *Hemigrammocapoeta*, the southeastern Asian-Indonesian ones to *Lobocheilus*.

Typhlogarra Trewagas, 1955. Valid.

Varicornis Ruppel, 1836. Valid, but only for African species; its East Asian presumed species belongs to *Onychostoma* (s.str. or subgenus *Scaphestes*: 14, 15), the western Asian ones to *Capoeta* (50).

Xenocyprioides Chen, 1982. Valid, according to description and illustration.

Zezera Jordan & Fowler, 1903. First synonymized to *Pungtungia* by Jordan & Hubbs (48).

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DYNAMICS OF THE CELL-CELL AND CELL-BASEMENTS MEMBRANE JUNCTIONS DURING SKIN ONTOGENESIS

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In the present paper, we have investigated the ultrastructural development of the epidermis and we focused our interest on the dermo-epidermal junction zone during golden hamster *(Mesocrycetus auratus)* development. During morphogenesis of the hamster tegument, there is dynamics of the basement membrane ontogenesis as well as of hemidesmosomal junctions complexes: 1) temporal, (a) the basement membrane precedes hemidesmosomal junction; (b) during hemidesmosome formation, hemidesmosomal attachment plaque could be detected before hemidesmosomal inner plate to be ultrastructurally built; 2) regionally the cervico-dorsal skin expresses earlier the components of dermo-epidermal junction, comparing with those of tail tegument which suggests different epithelio-mesenchymal interactions depending especially where regional dermal cells are originated from (the skin of Vertebrates is a segmented organ).

Interaction between dissimilar cell populations (for example epithelial-mesenchymal interactions) leads to the processes of 1) determination, 2) cytodifferentiation and 3) morphogenesis. It is generally presumed that each of the three processes above named represents a step in activation of specific gene expression (Bernfield M. R., and Wessels N. K., 1970, cited by Dawe C. J., 1976).

In connective tissue, extracellular matrix bears most of the stresses to which the tissue is subjected, while in epithelia, the cells themselves rather than the extracellular matrix bears most of the stress by means of strong intracellular protein filaments (components of the cytoskeleton) in connection with specialized intercellular junctions, specially desmosomal type.

The tissues that are subjected to severe mechanical stress, such as skin epidermis, gastrointestinal mucosa, myocardium, blood vessels etc., to function, have the cytoskeletal elements connecting a cell either to those of another cell or to extracellular matrix.

Anchoring junctions occur in two structurally and functionally different forms: 1) adherens junctions are connection sites for actin filaments; 2) desmosomes and hemidesmosomes are connection sites for intermedium filaments. Desmosomes connect intermedium filaments from cell to cell and hemidesmosomes connect intermedium filaments to basal lamina.

The skin is a vital organ which lines the body surface and repairs itself and which can illustrate very well the above general description.

The Vertebrate skin consists of a multi-layered cellular epidermis derived mainly from the surface ectoderm and an underlying dermis of mesodermal

origin. These two distinct parts of the skin meet at the epidermal-dermal junction zone (basement membrane) — a region where important morphogenetic and other influences operate during fetal and postnatal life.

Epithelial-mesenchymal interactions play an important role for the cytodifferentiation, histogenesis and normal development of epithelia including regional variations of epithelia during embryogenesis and postnatal.

Extracellular matrix plays dynamic roles in many tissue processes including development, tissue remodeling and repair, and the regulation of the cytodifferentiation and cell phenotype (10), (14). Extracellular matrix molecules appear early in development. Basement membrane is the first composite matrice to be formed during embryogenesis.

The present study is devoted especially to the preliminary electronmicroscopic investigations of the dermal-epidermal junction zone during hamster ontogenesis.

MATERIALS AND METHODS

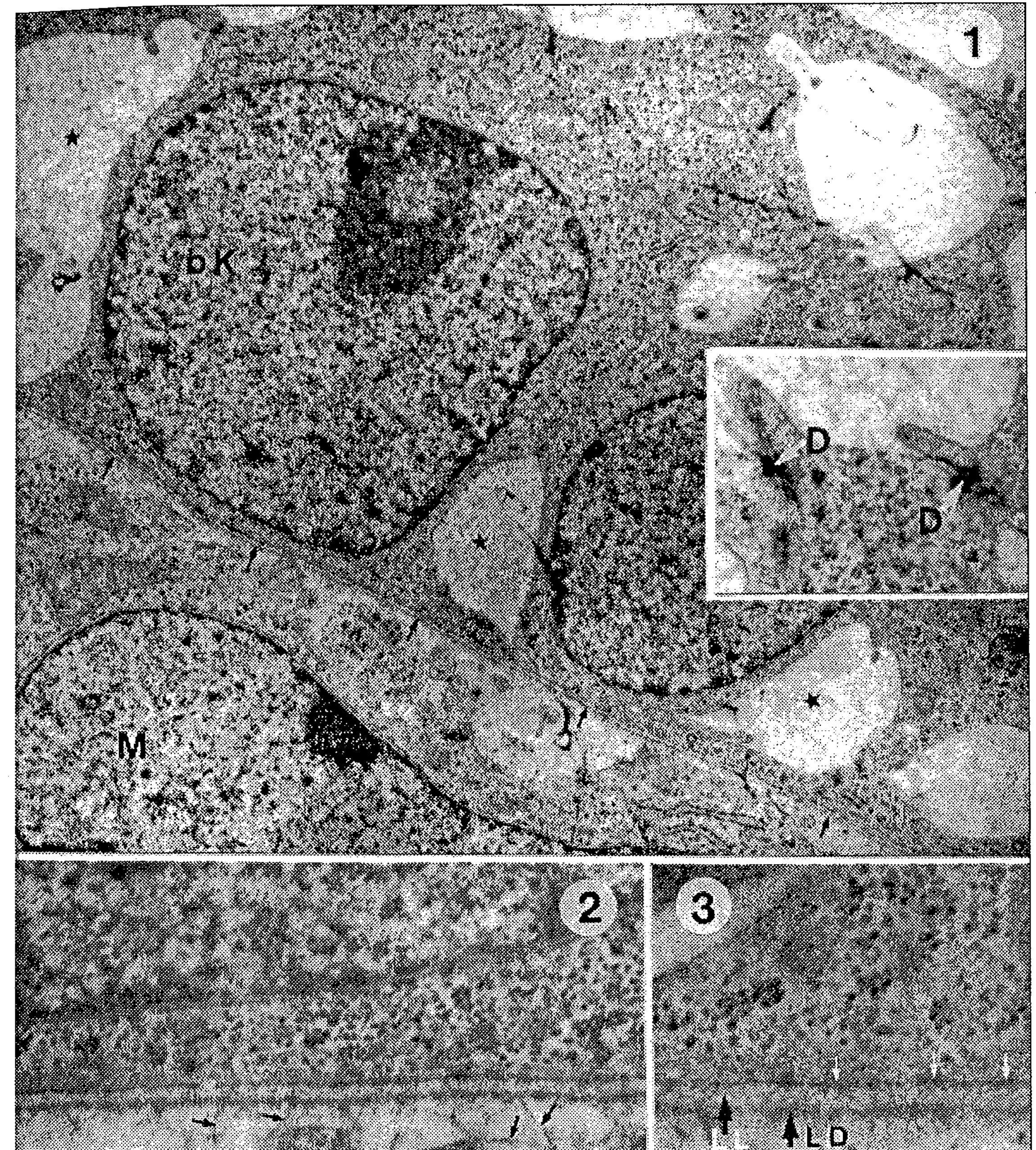
Small fragments of hamster tegument which belong to different body regions (cervico-dorsal, abdominal, tail etc.) taken at different stages of development (6 mm, 10 mm embryos, new born, 10 days postnatal and adult) were fixed in 2.5% glutaraldehyde and postfixed in 2% osmium tetroxide in sodium cacodylate buffer. Semithin sections were stained with 0.1% toluidine blue for light microscopical examination. 70–90 nm ultrathin sections were counterstained with uranyl acetate and lead citrate and investigated by means of an electronmicroscope operated at 60 kV.

RESULTS AND DISCUSSIONS

The present study describes the morphology of the hamster epidermis during morphogenesis with special references to the cell-cell and epithelial cell-basement membrane junctions.

When tail tegument of 10 mm length embryo was examined, we could distinguish an epithelium in way to participate in a primitive tegument formation, separated by a continuous basement membrane from subjacent mesenchyme. (Figs. 1–3). During this stage of embryo development, 1–3 nucleated epithelial cell layers can be observed. Basal keratinocytes are cuboidal, with large nuclei. Euchromatine is predominant and 1–2 large nucleoli can be counted. The uppermost cell layer becomes flattened.

The very large intercellular spaces are filled with an amorphous substance. Only seldom, microvilli originated from epithelial cells emerged into these inter-



ɡ. 1. — General view of the primitive tegument: basal epithelial cells (bK) with nucleolated nuclei are separated by a sal membrane (arrows) from subjacent mesenchyme (M). No hemidesmosome can be detected. Desmosomal juncns (D), (Fig.1, inset, × 16 900) are scarce and immature. Large intercellular spaces filled with amorphous substance (asteriscs) separate epithelial cells. (tail of 10 mm embryo), (× 10 700).

ɡ. 2. — Anchoring fibrils (small arrows) are in way to be formed. (tail of 10 mm embryo), (× 35 200).

ɡ. 3. — Small spots of dense material (small white arrows) mark the site where hemidesmosomal attachment plaque will be located; (L L = lamina lucida; L D = lamina densa), (tail of 10 mm embryo), (× 45 400).

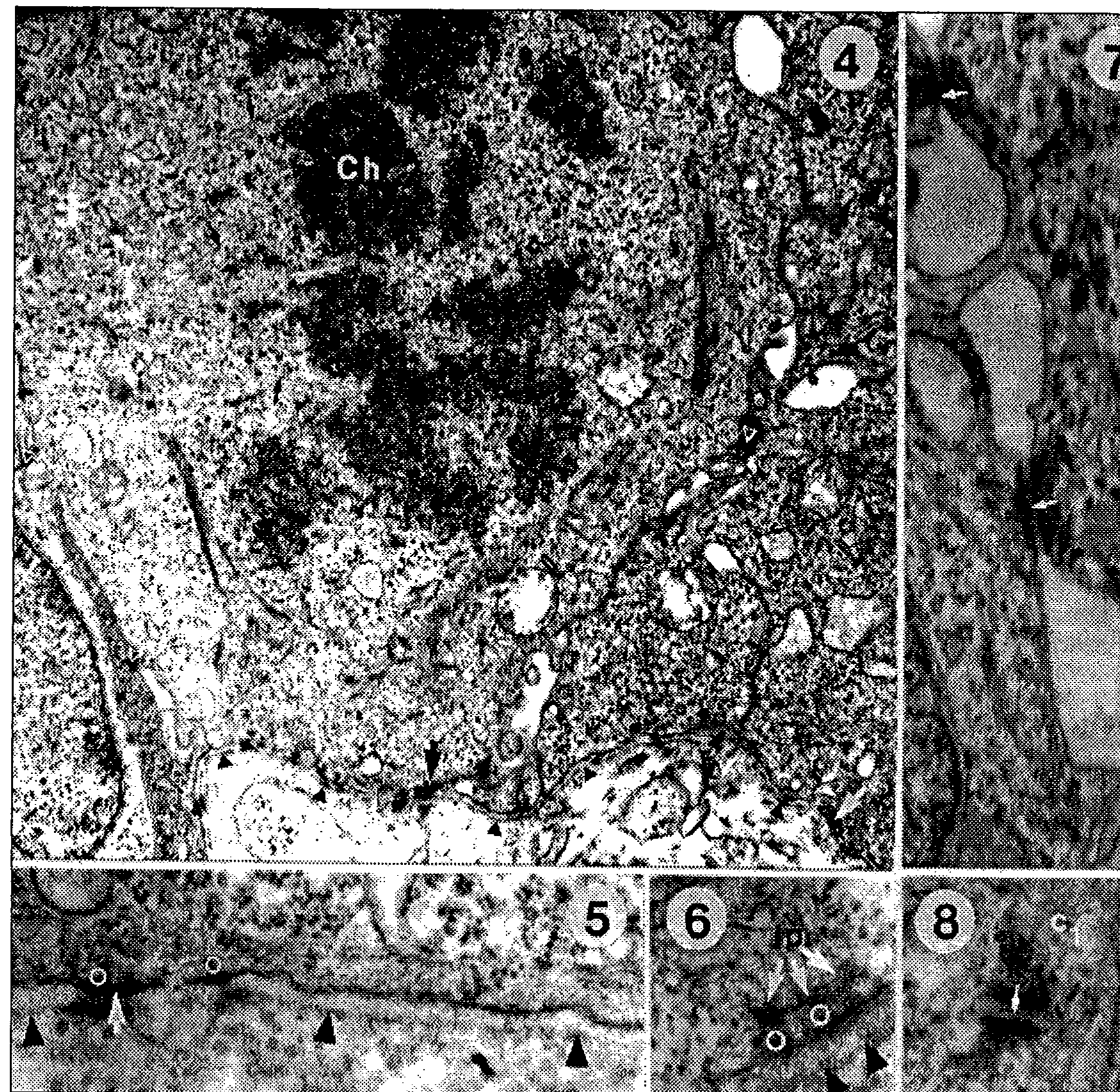


Fig. 4. — General view of the dermo-epidermal zone: a well-developed basement membrane can be seen (black tr gles). Immature and mature hemidesmosomal junctions are coexistent (large arrows). A basal keratinocyte during m sis (condensed chromatin — Ch — and microtubule — small arrows) can be observed. (cervico-dorsal epidermi 10 mm embryo), (× 10 400).

Figs. 5. and 6. — Details for immature (Fig. 5) and mature (Fig. 6) hemidesmosomes. In Fig. 5 white circles n hemidesmosomal attachment plaque. In Fig. 6, mature hemidesmosomes exhibit hemidesmosomal attachment pla (white circles) and hemidesmosomal inner plate (i p). (Fig. 5. × 33 400); (Fig. 6. × 45 500).

Fig. 7. — Mature desmosomes with middle lamellae (white small arrows) strongly connect adjacent keratinoc (cervico-dorsal epidermis of 10 mm embryo), (× 26 900).

Fig. 8. — An unaltered mature desmosome (white small arrow) is internalized into a keratinocyte (C_1). (cervico-do epidermis of 10 mm embryo), (× 60 100).

cellular spaces (Fig. 1). Intercellular focal adherens junctions or even desmosomal junctions connect adjacent epithelial cells. We observed also adherens junctions during in vitro and postgrafting human epidermis reconstruction (Mirancea N., et al., manuscript in preparation). Different of desmosomes which are associated with intermedium keratin filaments, Kaiser H. W. et al., (1993), detected that in human epidermis and gingival mucosa adherens junctions are associated with actin filaments, alpha-actinin and vinculin. It is interesting to underline that in contrast to desmosomes which are specific to adjacent epithelial cell junctions, adherens junctions mediate both cell-cell and cell-matrix contacts (7).

Desmosomes are rare and immature (Fig.1, inset). Cytokeratine intermedium filaments are scarce and these do not connect desmosomes. Although a basement membrane with a continuous lamina densa and anchoring filaments and moreover anchoring fibrils are visible (Figs. 2 and 3), ultrastructurally no hemidesmosomal junction could be detected.

Nevertheless, small spots of dense material attached to cytoplasmic face of basal plasma membrane suggest the places where hemidesmosomal attachment plaque will be developed (Fig. 3).

During the same stage of development, electronmicroscopic investigations of the cervico-dorsal skin showed 5–7 nucleated keratinocyte layers. Even during mitotic division, basal keratinocytes keep their polarity: they exhibit unaltered both desmosomal and hemidesmosomal junctions (Fig. 4).

Basement membrane is well developed (Figs. 4–6). It is interesting that at this stage immature (lacks inner hemidesmosomal plate; Fig. 5) and mature (both hemidesmosomal attachment plaque and inner hemidesmosomal plate, including intermedium filament connexion (Fig. 6)) hemidesmosomes are coexistant. Desmosomal junctions are mature and strongly connect adjacent epithelial cells (Fig. 7). Sometimes, complete cytoplasmic (internalized) desmosomes could be detected (Fig. 8). Except their uncommon location, ultrastructurally the complete cytoplasmic desmosomes are very similar to those of the surface desmosomes. Such kind of complete cytoplasmic desmosomes was detected especially in case of neoplastic cell proliferation and they should be distinguished from cytoplasmic half desmosomes. Shabana et al. (1994, a and b) demonstrated that acrylamide treatment of cultured rat keratinocytes induces intermediate filament disturbance and cause actively complete cytoplasmic desmosomes. The trypsinization or lowering calcium level in cell culture medium induces internalization of half desmosomes by endocytotic vesicles (8, 19 a). Internalization of complete desmosomes in case of malignant cells strongly correlates with the requirement to reduce intercellular adhesion in order to achieve a high degree of freedom — an important aspect of the neoplastic invasive cell behaviour. It is

difficult to speculate why occasionally complete cytoplasmic desmosomes are detectable during normal epidermal morphogenesis.

Ultrastructurally, the skin of the new born hamster is comparable to the postnatal and mature hamster skin. Multilayered epidermis with desquamation was observed. The histological organization of new born hamster skin is shown in Fig. 9. Above the dermis, the basal layer of cells is superimposed by layers of the stratum spinosum, a distinct keratohyaline cell zone and a thin layer of cornified cells.

The dermo-epidermal junction structures strongly connect epidermis to the subjacent mesenchyme: a continuous basement membrane (Fig. 10), numerous mature hemidesmosomes (Figs. 11 and 12). Vigorous anchoring fibrils, found immediately beneath the lamina densa, preferentially in the vicinity of hemidesmosomes, could be observed (Figs. 11 and 12).

The dermo-epidermal junction is a key structure in the skin. Hemidesmosomes and anchoring fibrils are essential structural components of the dermo-epidermal junctions (1), (9), (15), (17), (18).

During morphogenesis of the hamster tegument, there is a dynamics of the basement membrane ontogenesis as well as hemidesmosomes junctional complexes: 1) temporal, (a) the basement membrane appears before hemidesmosomal junctions; (b) during hemidesmosome ontogenesis, hemidesmosomal attachment plaque (adjacent to plasma membrane) could be detected before hemidesmosomal inner plate (which is more cytoplasmic located) too be formed; 2) regionally the cervico-dorsal tegument expresses earlier the components of dermo-epidermal junction comparing them with those of tail tegument. The skin of Vertebrates is a regional (segmented) organ (3).

It is particularly interesting to remark that ultrastructural, defective hemidesmosomes for hemidesmosomal inner plate (and consequently, defective for the connexion with cytokeratine filaments) from a malignant human keratinocyte clone which grows invasively (Mirancea N. et al., manuscript in preparation) strongly remember of the immature hemidesmosomes during natural hamster epidermal morphogenesis.

How much important are anchoring fibrils for the stability of dermo-epidermal junction is deduced from numerous papers which reported that patients with dystrophic epidermolysis bullosa, a disease in which anchoring fibrils are scarce or totally lacking and the skin blisters drastic and excessive (1), (18). Where do anchoring fibrils originate from? In an experimental study, Regauer et al., (1990), reported that the type VII collagen of human skin as major structural component of anchoring fibrils is synthesized by keratinocytes. However, Briggaman et al., (1971, cited by Konig A., et Bruckner-Tuderman L., 1991) have first shown that both dermal and epithelial components are required for the formation of anchoring fibrils in reconstructed skin. Experimentally, Konig A.,

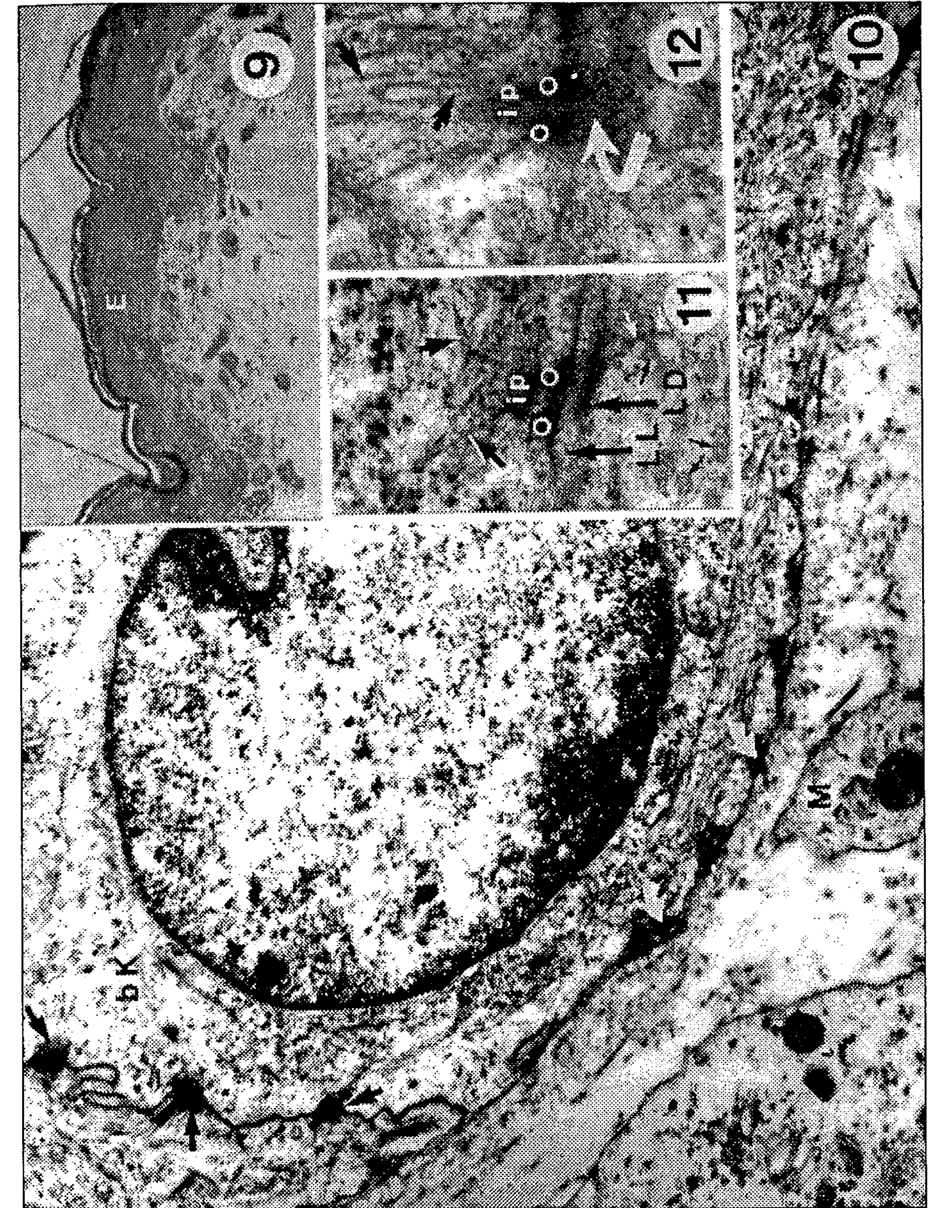


Fig. 9. — Histologic aspect of the cervico-dorsal epidermis (E) of new born hamster. (objective x 40).
Fig. 10. — A continuous basement membrane with numerous hemidesmosomes (white curved arrows) strongly connects basal keratinocytes (bK) with subjacent mesenchyme (M). Black arrows mark desmosomal junctions between adjacent basal keratinocytes. (cervico-dorsal epidermis of new born hamster), (x 22 000).
Figs. 11. and 12. — Detailed ultrastructural aspect of the mature hemidesmosome: the hemidesmosomal attachment plaque (white circles) and more cytoplasmic located inner plates (i p) which strongly connect hemidesmosomes to well-developed intermedium filaments (black arrow heads). Moreover, anchoring filaments (white curved arrow) and anchoring fibrils (small white arrows) which connect basement membrane (L L and L D) to the collagen fibrils (black small arrows) from the dermis are well represented. (cervico-dorsal epidermis of new born hamster). (Fig. 11, x 49 000; Fig. 12, x 65 000).

Fig. 13. — In the uppermost layers of epidermis, a transitional keratinocyte (T K) exhibits "half desmosomes" (h D) which still keep a good connexion with long tonofilaments. (cervico-dorsal epidermis of new born hamster). (× 28 000).
Fig. 14. — Large and polymorphic keratohyaline granules (khG) are strongly connected with tonofilaments (arrow). (cervico-dorsal epidermis of new born hamster). (× 48 600).

and Bruckner-Tuderman L., (1991) demonstrated that collagen type VII is synthesized early by keratinocytes, but structural anchoring fibrils detection needs epithelio-mesenchymal interactions (15).

Within dermis, mesenchymal cells are very rich in rough endoplasmic reticulum and Golgi complexes which suggest a high biosynthetic activity in order to build an extracellular matrix.

In stratum granulare, large keratohyaline granules could be connected with tonofilaments (Fig. 14). Often, they are polymorphic (electrondense and electronlucent areas are coexistant in the same keratohyaline granule).

Using double immunogold labelling, Manabe and O'Guin (1994) showed that in case of heterogenous electrondensity of keratohyalin granules from stratum granulare of nonfolicular epithelia (mouse dorsal tongue epithelium), the electrondense areas correspond to the trycohyalin and the marker for the pro-fillagrin is located in electronlucent area.

During the normal process of cornification, drastic ultrastructural alterations of desmosomes took place. First of all, at the transitional zone between stratum granulare and stratum corneum ("transitional keratinocytes") so-called "half desmosomes" are observed (Fig. 13). For the desmosomes between corneocytes Mils V. et al., (1992) proposed them the term "corneodesmosomes".

A marker for the terminal differentiation keratinocytes is the cornified envelope that forms adjacent to the plasma membrane of the uppermost granular keratinocytes and stratum corneum as a result of an enzymatically covalent cross-linkage of proteins and lipids (a highly insoluble product which plays an important role in barrier function of water loss through epidermis (4), (6), (12), (16)).

In spite of the fact that there seems to be an alteration of the lamellar body morphology and content delivery, which partly comes from the fixation with osmium tetroxide (21), we observed that the upper cell layer of stratum granulosum is able to form saccular invagination and the content of (abnormal) lamellar bodies is extruded within the dilated interdesmosomal regions at the interface stratum granulosum/ stratum corneum.

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STRUCTURAL CHANGES INDUCED BY COPPER INTOXICATION IN *ANODONTA CYGNAEA* LINNÉ (*LAMELLIBRANCHIATA*) GILLS AND HEPATOPANCREAS

VIORICA MANOLACHE*, VIRGINIA POPESCU-MARINESCU**, MARIA NĂSTĂSESCU*, CARMEN MARINESCU**

In *Anodonta cygnaea* species there were followed the changes produced by copper intoxication (from copper sulfate) in gills and hepatopancreas. Among these ones, the hepatopancreas especially proved to be the most sensitive to the pollutant action, even in very small doses.

Heavy metals represent important sources of pollution for surface waters, due partly to their toxicity and, on the other hand, to their stability.

This aspect is expressed by pertubations of the biological balance with multiple implications within the aquatic ecosystem.

In the present paper we have followed copper influence on gill and hepatopancreas in *Anodonta cygnaea*.

Copper is part of a certain enzyme structure as well as of hemocyanin in molluscs. Its toxicity results from its interaction with cell membranes which are labilized by a lipoperoxidative process (2), (3).

MATERIAL AND METHODS

Anodonta cygnaea specimens were intoxicated with various concentrations of copper (from $CuSO_4$ put into water). The doses used were of 0.05 and 0.3 mg/l for 72 h. After that period the animals were killed and the gills and the hepatopancreas were taken. These pieces as well as those derived from controls were fixed in Bouin and calcic phormol, then processed according to classic histological techniques. The sections were coloured by current histological and histochemical techniques.

RESULTS

Anodonta cygnaea gill is of W form, upward and downward branches being connected by bridges of connective tissue (Fig. 1).

Each of ctenidial comes in touch either with mantle or with visceral mass.

Epithelium covering the gill is unistratified and composed of ciliated cylindrical cells; the cilia have various dimensions. Cell nucleus is ovoid, cen-

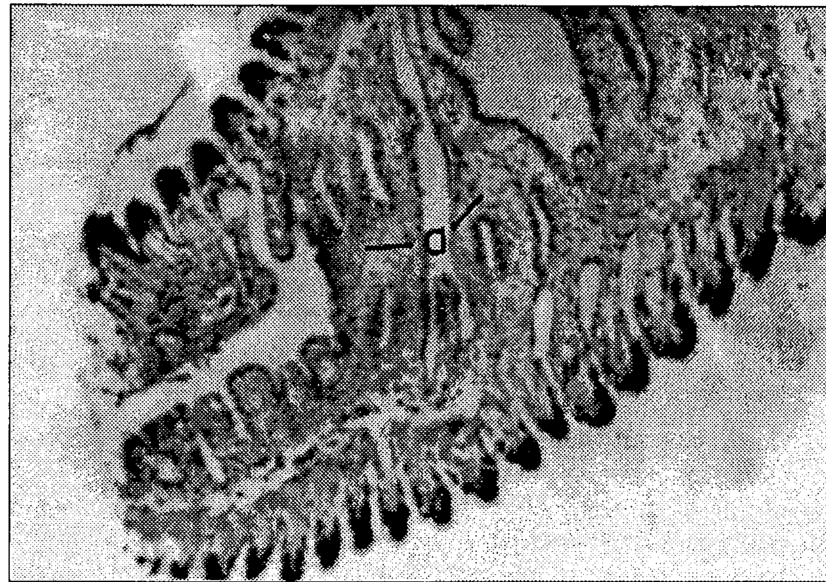

Fig. 1. — *Anodonta cygnaea,* gill control, 40 × 0.65: a) connective tissue bridge.

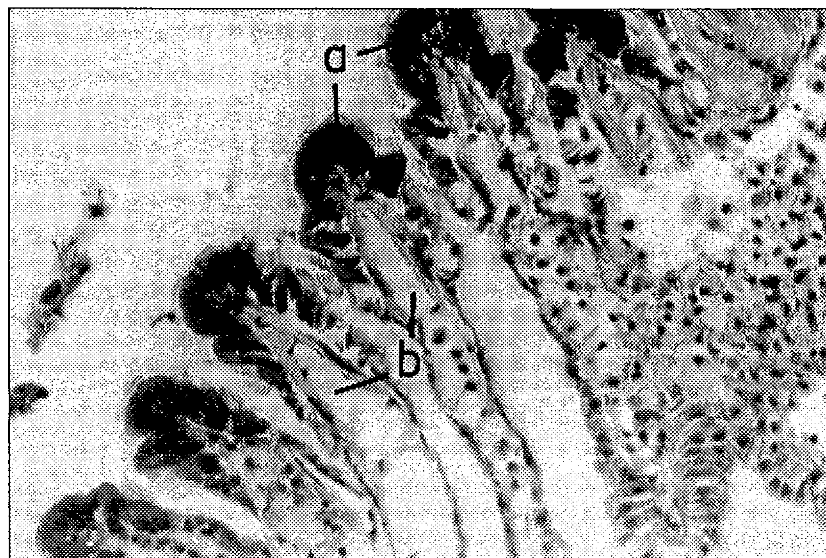

Fig. 2. — *Anodonta cygnaea,* gill intoxicated with 0.05 mg/l copper dose, for 72 h. 3.20 × 0.10: a) gill epithelium; b) blood vessel; c) connective tissue.

trally placed and slightly chromatic. Sometimes, among ciliated cells mucous cells are also observed. At epithelium basis there is a chitin layer of various thickness. The chitin may be interrupted by muscles that cross the filament from one branch of gill to another one.

Muscle also exists in gill axis at the filament basis and within interfoliar and interfilamentar junctions.

In gill axis there is also a connective tissue within which one may observe also connective cells, collagen fibres, blood cells (erythrocytes, amoebocytes).

The muscles existing in the axis are disposed into all directions (5).

The animals treated with a dose of 0.05 mg/l copper show no obvious changes either in the gill epitehelium, or in the existing blood vessels (Fig. 2). Seldom there are pycnotic muclei. Also, a slight hypertrophy of cylindrical cells of gill epithelium was noticed. Sometimes the connective tissue pushes gill epithelium, such as among epitheliad cells one may see ameobocytes. Generally, connective cell nuclei are pycnotic and ameobocytes of eosinophilic granulations appear hypertrophied with a pycnotic nucleus as well.

At a dose of 0.3 mg/l copper all of structural components of gill were affected, gill epithelium appears completely disorganized and here and there it is even completely destroyed (Fig. 3 and 4). Cell limits disappear. Nuclei are either totally deformed or pycnotic. Disorganization leads to cell stratification in certain areas.

Sometimes we have noticed that the connective tissue is rarefied. Amoebocytes and other blood cells as well as connective cells are little represented.

The musculature as well as another connective tissue components in gill axis are disintegrated. Blood vessels are altered and limits disappear. Their endothelium is destroyed, in some areas one may observe blood vessel fusion.

We point out that 0.05 mg/l copper dose did not always cause pronounced changes in the gill structure.

A stronger degradasion was produced by 0.3 mg/l copper where changes are obvious.

Hepatopancreas in *Anodonta cygnaea* controls are under the form of digestive diverticles (Fig. 5) surrounding the stomach, and their structure was described in the same species by Manolache and collab. (7).

The specimens treated with different doses of copper react differently depending on the amount of pollutant used.

At 0.05 mg/l copper the great majority of hepatopancreas tubules (Fig. 6) are disintegrated. Dark cells as well as the clear ones do not maintain their shape, cell limits disappear, nuclei are very chromatic or pycnotic. Instead of cells, vacuoles appear where 2–3 pycnotic nuclei are present. In areas where hepatopancreas tubules are disrupted, the tubule interior is occupied by a connective tissue within which a high number of amoebocytes of extremely diformed and superposed eosinophilic granulations are present.

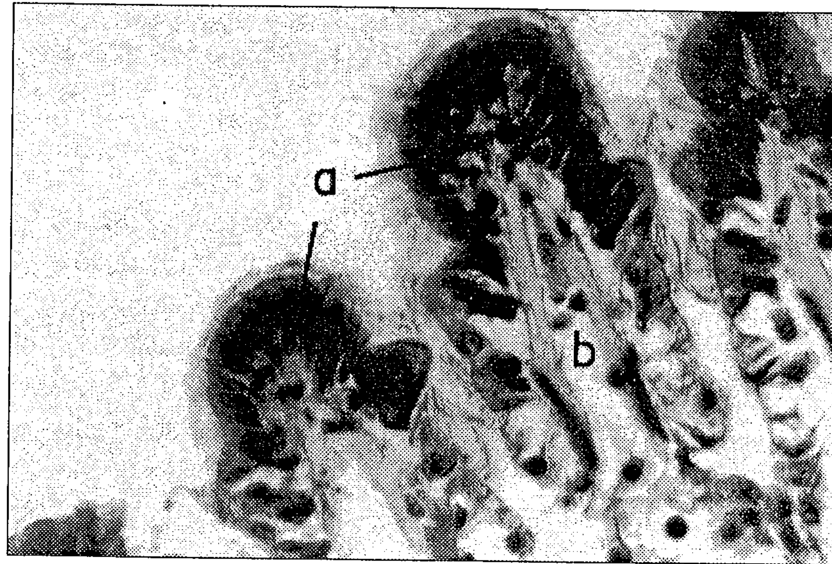

Fig. 3. — *Anodonta cygnaea,* gill intoxicated with 0.05 mg/l copper dose, for 72 h, 10 × 0.20: a) gill epithelium; b) blood vessel.

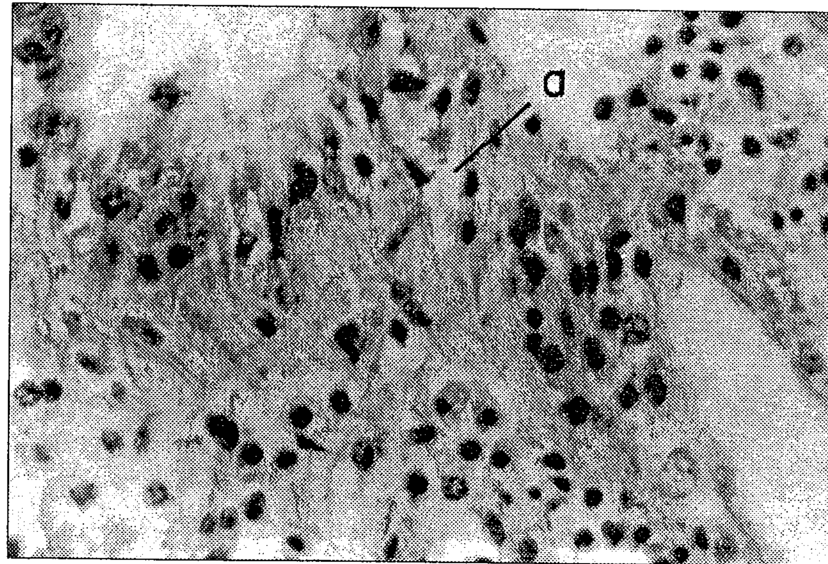

Fig. 4. — *Anodonta cygnaea,* gill intoxicated with 0.3 mg/l copper dose, for 72 h, 3.20 × 0.10: a) gill epithelium disorganized.

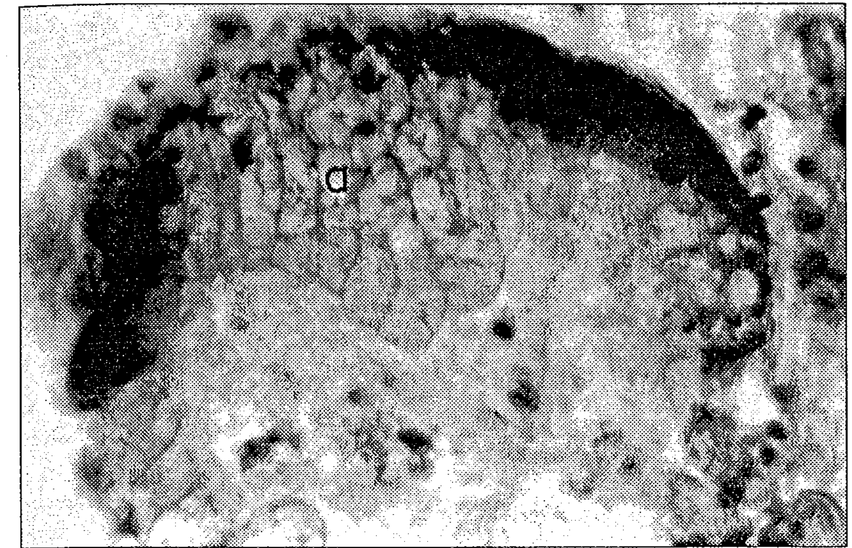

Fig. 5. — *Anodonta cygnaea,* hepatopancreas control, 20 × 0.40. Hepatopancreas tubules with: a) clear cells: b) dark cells.

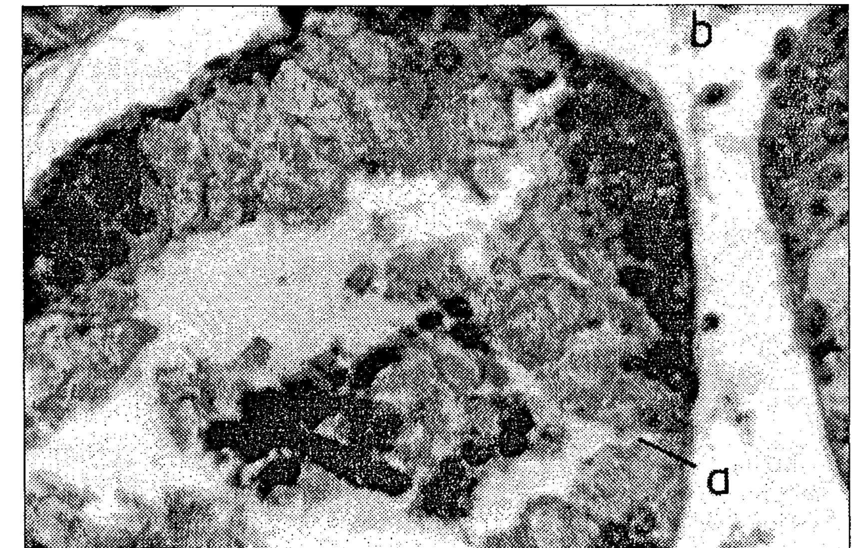

Fig. 6. — *Anodonta cygnaea,* hepatopancreas intoxicated with 0.05 mg/l copper dose, for 72 h, 20 × 0.40: a) ephitelium disintegrated; b) connective tissue.

The connective tissue between tubules is rarefied. Among collagen fibres few connective cell nuclei are noticed, as well as amoebocytes displayed in stacks. The nuclei of these cells are destroyed.

At 0.3 mg/l copper (Fig. 7 and 8) a very pronounced hypertrophy of dark and clear cells is noticed. Cell nuclei sometimes enlarge enormously, other times they appear pycnotic. In case epithelium is disintegrated, the connective tissue penetrates its interior. Connective and blood cells of this connective tissue are rarefied. Sporadically, only a few pycnotic nuclei are noticed. Also, collagen fibres are rare.

We point out that the specimens intoxicated with a lower dose of copper show a pronounced degradation of hepatopancreas tubules compared to the specimens treated with a higher dose. Also, the connective tissue between tubules reacted differently, so in animals intoxicated with a higher dose of copper connective cells are much more affected, being very rare.

DISCUSSION AND CONCLUSIONS

Literature data concerning structural changes induced by heavy metal intoxication of gills and hepatopancreas are scarcely enough (1), (3), (10).

As we also have pointed out, and other authors did, more pronounced changes are seen especially at the hepatopancreas level.

Sarasquette M. C. et al. (10) have found histological alterations caused by copper and cadmium at the hepatopancreas level.

Many of intervertebrates were found to have the ability to form intracellular, mineral deposits, forming spherical granules (11). These granules contain calcium and phosphorus and recently heavy metals were also detected within them (4).

Even less invetigations were made as concerns the gills (1), (3).

Studies of manganese and iron influences on gills, mantle and digestive gland were performed in *Anodonta cygnaea* species by Nicole Lautié and coworkers (6). Gill epithelia accumulated a high amount of these metals.

Copper action on the structures analysed by us has been little studied. In many cases, our observations confirmed the literature data concerning the action of certain heavy metals on different animal tissues.

We point out that in analysed specimens in case of hepatopancreas we sometimes noticed a more pronounced degradation of tubules epithelia even with a lower dose intoxication. Also, it was observed a pronounced change in the connective tissue between the hepatopancreas tubules that appeared very degraded.

As concerns gill epithelium, very pronounced changes appear only at a higher dose, of 0.3 mg/l.

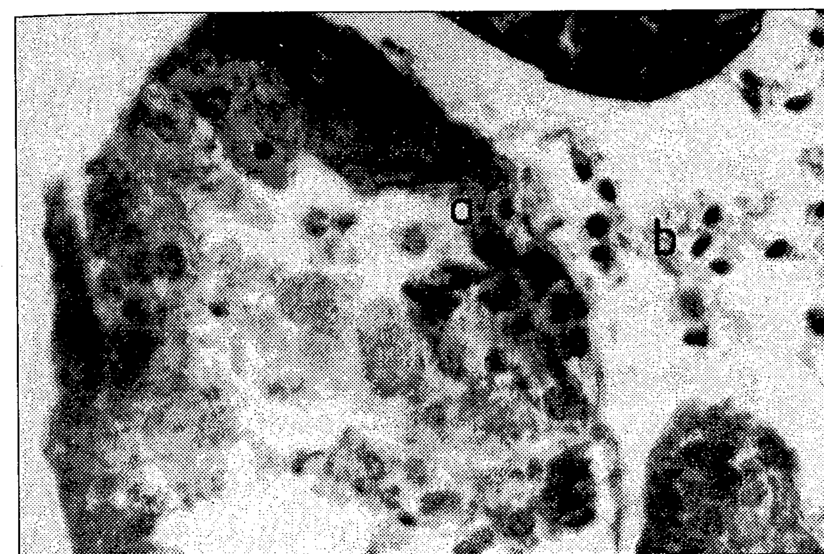

Fig. 7. — *Anodonta cygnaea*, hepatopancreas intoxicated with 0.05 mg/l copper dose, 20 × 0.40: a) epithelium disintegrated; b) connective tissue.

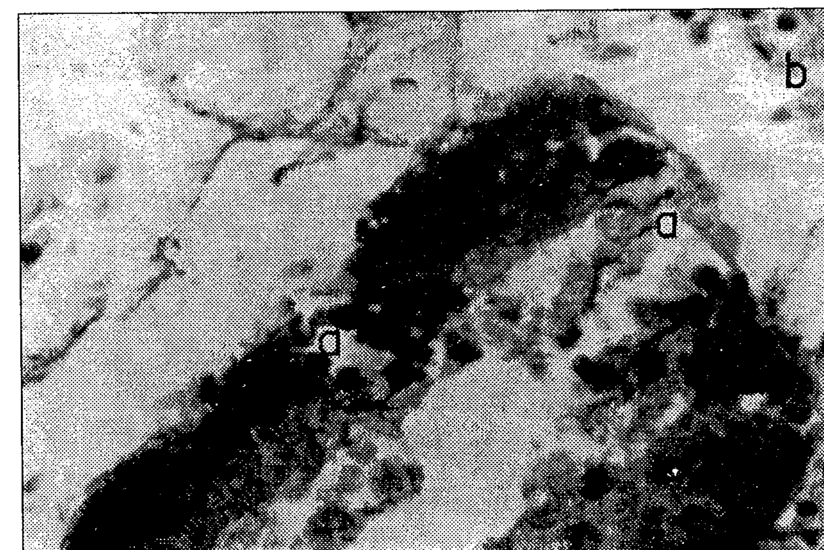

Fig. 8. — *Anodonta cygnaea*, hepatopancreas intoxicated with 0.3 mg/l copper dose, 20 × 0.40: a) epithelium disintegrated b) connective tissue.

In case of copper intoxication we have noticed amoebocyte infiltration in gill epithelium as well as a higher number of them in the connective tissue surrounding the hepatopancreas.

These remarks are similar to those of other authours (4), (7), (8), (9), who noticed accumulation of amoebocytes in a high number both in various epithelial tissues and in the connective tissue around them. In these situations amoebocytes have a role in bioaccumulation of various heavy metals as well in tissue detoxification.

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DYNAMICS OF THE ACTIN CYTOSKELETON IN RENAL CELLS FROM THE AFRICAN GREEN MONKEY (VERO) AND IN HUMAN PULMONARY FIBROBLASTS (ICP23 DIPLOIDS) INFECTED WITH MEASLES VIRUS

VIORICA MANOLACHE, MARIANA ORĂŞANU, NICOLETA TELETIN, MARIANA GĂLEŞANU, VALERIA CLUCERESCU, DOINA PETRAŞINCU, LILIANA BABEŞ

Alterations of F actin in cell cultures VERO and ICP23 infected with the measles virus are described in comparison with control cells at 24 and 48 hours after growth initiation. Modification of cellular shape, intercellular contact structures as well as actin filament and stress fiber disorganization could be noticed.

Actin, one of the cytoskeletal proteins, can be found in most eukaryotic cells. F actin, the polymerized form of G actin, can be observed within the cells either as long, thin filements, or as thick filaments that constitute stress fibers.

Along with the other cytoskeletal components, actin has a role in maintaining the cellular shape, in cell division cycles, in cell motility, in the formation of intercellular junctions, and, in the case of cell cultures in cell attachment to the substrate. These parameters may be affected by viruses entering the cells, by their proliferation and elimination.

The measles virus, once inside the cell, incorporates the actin of the host cell into its own structure and thus produces morphological modifications at the level of the actin cytoskeleton (1, 3, 4).

In this paper we studied the arrangement of actin microfilaments in normal cell cultures – the continuous line VERO (renal cells from the African green monkey) and the diploid line ICP23 (human embryonic pulmonary fibroblasts) and their modification in cells infected with measles virus.

MATERIALS AND METHODS

Cell cultures: The VERO line was grown in MEM (minimal essential medium) with 5% fetal serum and the human pulmonary fibroblasts (ICP23) (7) in BEM (basal Eagle medium) with 10% fetal serum. Both cultures were maintained in Eagle medium with 1% fetal serum.

Wilde measles virus, strain Edmonston B, was maintained on VERO. The cultures were infected with virus upon formation of complete monostrate (48 hours for the VERO line and 72 hours for ICP23).

Visualization of the F actin filaments. Cells were grown on slides set on Linbro plates (∅7 cm) washed with saline phosphate buffer fixed 15′ in 3.8% paraformaldehyde, washed again in saline phosphate buffer and permeabilized with Tween X-100, 0.1% for 4′. Cells were then again washed with saline phosphate buffer and stained with the conjugate phalloidin-FITC (Sigma), in which they were incubated for 30′ at room temperature in the darkness. Then, the slides were set in cedar oil and the samples were visualized in epifluorescence under the microscope LEITZ-LABOR LUX Sc (thanks to Dr. Dan Ionescu). The photographs were made on film AGFAPAN-700.

The actin cytoskeleton was studied at the cells from the VERO line at 24 and 48 hours, until the monostrate was perfectly confluent. After infection with virus, the aspect of cytoskeleton was followed at 24 and 48 hours post infection. For the normal cells of the VERO line the cytoskeleton was studied by (5).

In the case of normal ICP23 diploids the evolution of the actin cytoskeleton was studied at 6, 24 and 48 hours and the modifications due to viral infection at 24 and 48 hours after infection.

RESULTS

CONTROL — THE CONTINUOUS VERO LINE

After 24 hours, the cells are completely laid down; they have numerous contact zones among them and they are adherent to the substrate. F actin appears as thin fibers that cross the cytoplasm of the cells and adhere in close proximity of the plasmalemma. At the intercellular contact zones, regions with intense positive staining can be noticed, proving the presence af actin in the structures of intercellular contact (panel 1, Fig. 1).

After *48 hours* the actin fibers appear to be thicker, and stress fibers that cross the cytoplasm in all directions can be visualized. The peripheric actin, which is involved in the structure of intercellular contacts, is more obvious, so that the cells can be easily distinguished (panel 1, Fig. 2).

CELLS OF THE CONTINUOUS LINE VERO INFECTED WITH MEASLES VIRUS

24 hours after the infection, the monostrate is unequally affected. Some cells modify their shape and the actin cytoskeleton is partially altered. In some zone the actin filaments maintain their integrity and look the same as the control filaments. In other regions the filaments are broken (panel 1, Figs. 3, 4). Towards the end of the 24 hour interval the infected cells maintain partial contact with normal ones and the center of the infected cells is deprived of filaments.

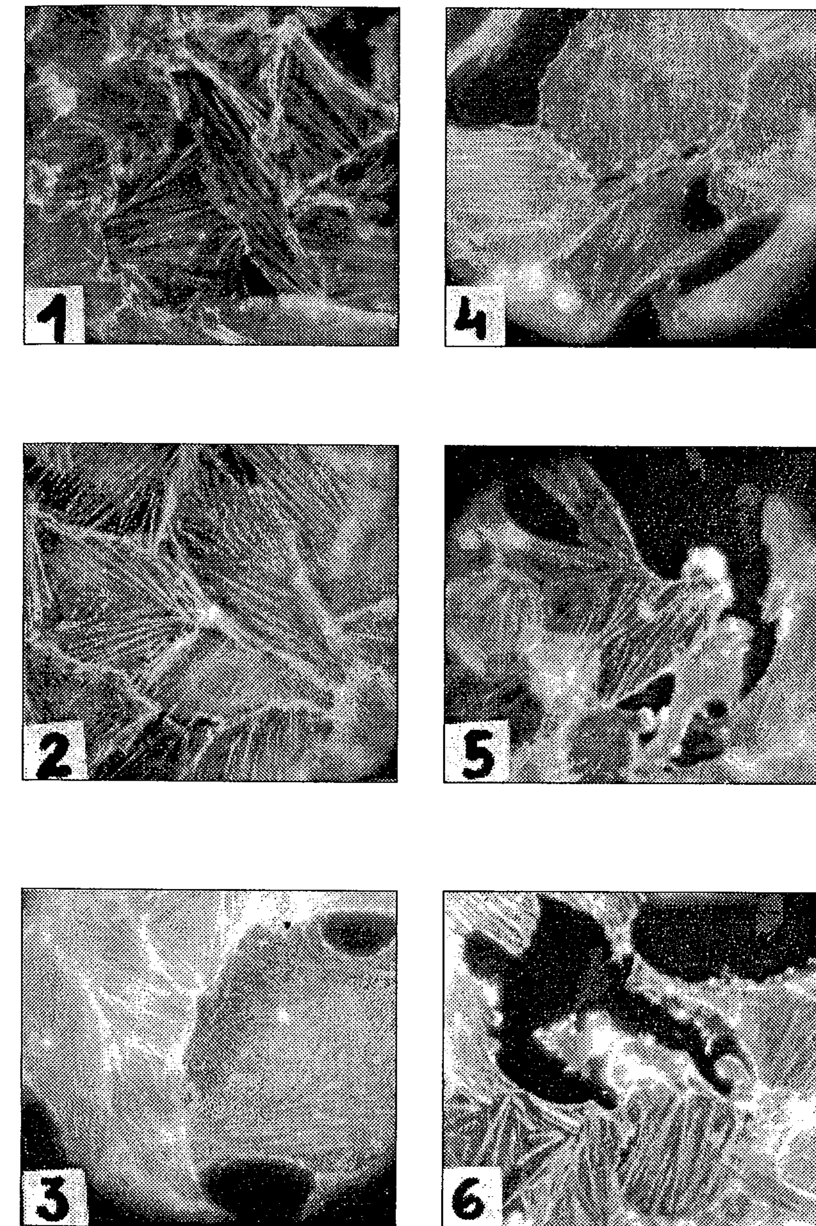

Plate I — The continuous line VERO: Control and cells infected with the measles virus.
Aspect of cells and actin fibers in:
Fig. 1. — Control VERO culture at 24 hours.
Fig. 2. — Control VERO culture at 48 hours.
Figs. 3, 4. — VERO culture infected with measles virus, 24 hours after infection.
Figs. 5, 6. — VERO culture infected with measles virus, 48 hours after infection.

Over the remaining cytoplasm the actin filaments appear broken. At the cell's edges, the positive staining proves the presence of normal actin filaments.

After *48 hours* the measles virus produces more obvious alterations. The number of infected cells grows. The cells modify their shape and become oval, or with irregular shape. Intercellular contacts disappear and intercellular spaces are bigger. The alterations of the actin filaments are also more obvious. Thus, they appear broken, finely granulated or like dense blocks that can protrude at the cell surface (panel 1, Figs. 5, 6).

CONTROL-HUMAN PULMONARY FIBROBLAST CELLS-DIPLOID LINE ICP23

The cells of control cultures develop more slowly, and for this reason the observation intervals were bigger. 6 hours after trypsinization the cells are still isolated and they begin to adhere to the substrate. They still have an irregular shape, they present very thin and long processes and within the cells the F actin appears finely granulated (panel 2, Fig. 1).

After *24 hours*, the density of the cells is bigger, the cells begin to come into contact with each other, the number of their processes grows and in the cytoplasm, on the finely granulated background, thin actin filaments appear (panel 2, Fig. 2). The filaments are quite visible in the star-shaped cells, where they reach even the finest processes.

After *48 hours,* the F actin fibers are very numerous and visible. They are parallel among themselves and with the long axis of the cells being present even in the finest processes (panel 2, Fig. 3).

CELLS OF THE ICP23 LINE INFECTED WITH THE MEASLES VIRUS

24 hours post infection, an important part of the cells are affected by the entrance of the virus. The cells retract their processes, which often break down. In the infected cells actin loses its fibrillar arrangement and appears like dense blocks with irregular shape on a finely granulated background (panel 2, Fig. 4).

48 hours post infection the damaging of the cells produced by the measles virus is even more obvious. The processes are no longer attached to the substrate and they often appear superimposed on other cells or processes. In the cells where filaments can still be seen, they are no longer perfectly straight but have a rather sinuous shape (panel 2, Fig. 5). As the processes tear themselves apart, the F actin filaments seem to retract from them (panel 2, Fig. 6).

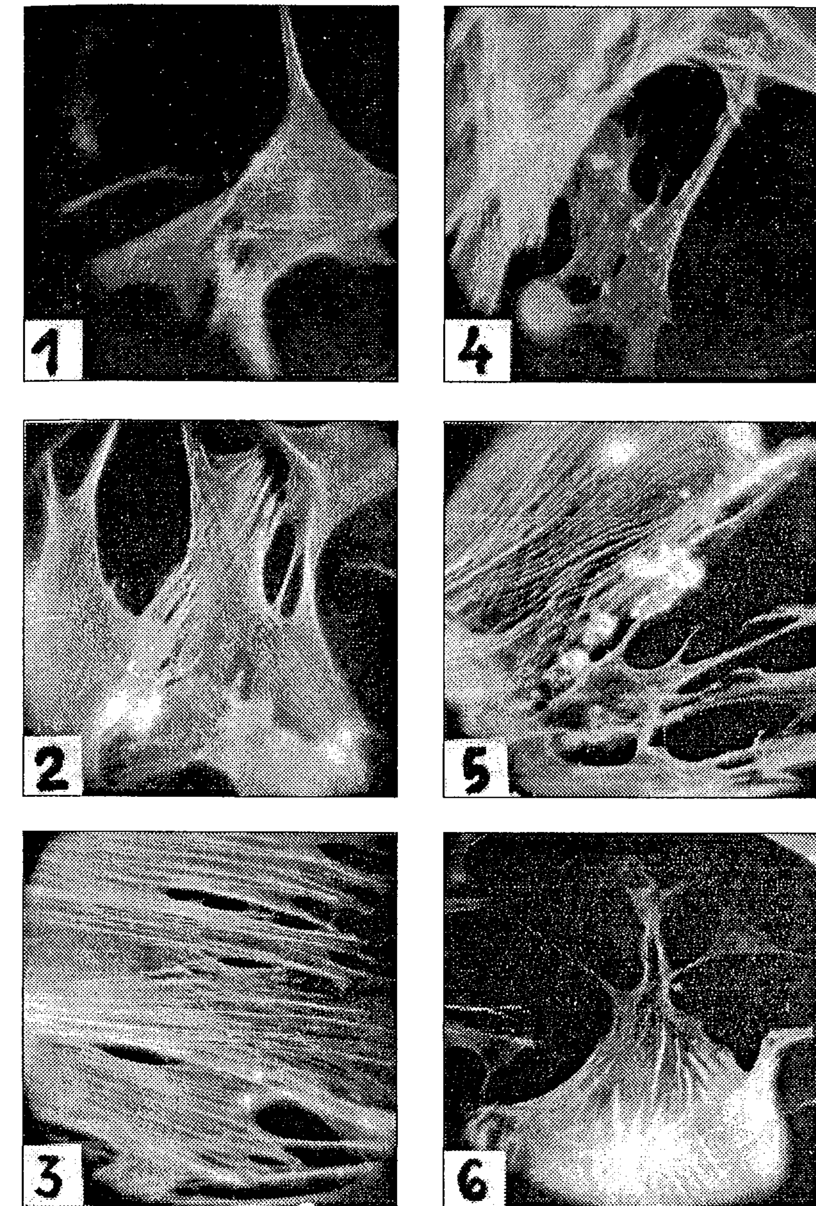

Plate II — The human embryonary pulmonary fibroblast culture-the diploid line ICP23: Control and cells infected with the measles virus.
Aspect of fibroblasts and F actin in:
Fig. 1. — Control diploid line ICP23 at 6 hours.
Fig. 2. — Control diploid line ICP23 at 24 hours.
Fig. 3. — Control diploid line ICP23 at 48 hours.
Fig. 4. — Cells of the ICP23 line 24 hours post infection.
Figs. 5, 6. — ICP23 Fibroblasts 48 hours post infection.

THE INFLUENCE OF OVIDUCTAL CONDITIONED MEDIUM ON BOAR SPERM MOTILITY AND CAPACITATION

ANCA OANCEA*, OANA CRĂCIUNESCU*, MARIA CALOIANU*,
ŞT. MANTEA**, MARIANA OPREA*

We have investigated the influence of oviductal medium on boar sperm motility and capacitation. Sperm from three boars was incubated in either isthmic or ampullary conditioned medium or control medium for 10 h. Sperm motility and capacitation were assessed at 10 minutes, 4 h and 6 h. In a second experiment, the glycosaminoglycan (GAG) content of the two types of oviductal conditioned medium was quantified by a specific method using Alcian Blue. Then, the samples of oviductal conditioned medium were treated with chondroitinase ABC and their ability to maintain the sperm motility and to promote the capacitation was assessed. Our results have indicated that the oviductal explants secreted chondroitin sulfate-type glycosaminoglycans in the culture medium, which are responsible for the maintenance of motility and for the capacitation of boar spermatozoa.

The mammalian oviduct provides the microenvironment suitable for the transport and final maturation of gametes, fertilization and early embryonic development. Oviductal fluid is composed predominantly of plasma derivatives (1), but it also contains nonserum macromolecules that are synthesized and secreted by the oviductal epithelium.

Oviductal secretory glycoproteins have been identified and characterized in mice (2), hamsters (3), rabbits (4) and sheep (5), but only a few studies were performed in pigs or humans.

It is also known that bovine oviductal fluid capacitates spermatozoa and sustain their mobility *in vitro* (6). The bovine oviductal fluid contains some proteins and glycosaminoglycans (7) synthesized by the oviductal epithelium.

The present study was undertaken to determine whether boar sperm capacitation and motility are affected by sow oviductal conditioned medium of oviductal explants from different regions of the oviduct. We also investigated the type and the amount of glycosaminoglycans secreted in the conditioned oviductal medium and if they are responsible for the sperm capacitation and motility.

MATERIALS AND METHODS

CULTURE OF OVIDUCTAL EXPLANTS

Oviducts were collected from 3 sows with normal estrous cycle that were sacrified at the slaughter house. The oviducts were trimmed of surrounding tissue and divided into ampulla and isthmus. Each region was cut open longitudi-

REV. ROUM. BIOL.–BIOL. ANIM., TOME 42, N° 1, P. 57–61, BUCAREST, 1997

nally and dissected into 4–5 mm pieces of tissue containing epithelium, stroma and muscle layers. To maintain intercellular associations and stimulate *in vivo* conditions the epithelium was not removed. Ten pieces of tissue were cultured in 5 ml of RPMI 1640 (Sigma Chemical Co.,/Dulbecco's Minimum Essential Medium) supplemented with 10% bovine fetal serum in 45 x 20 mm Petri dishes, at 37°C in humidified 5% CO_2: 95% air. After 18 h, 2 ml of fresh medium was added to each dish and explants were cultured for an additional 24 h. Conditioned culture medium was centrifuged (2000 x g, 30 min.) and the supernatant was recovered. In each sample, the protein concentration was determined by the method of Lowry.

EFFECT OF OVIDUCTAL CONDITIONED MEDIUM ON SPERM MOTILITY AND CAPACITATION

Semen was collected from three boars by use of an artificial vagina. Aliquots of semen from each boar were pooled in 10 ml of control medium (8). Sperm was washed by centrifugation for 10 min., at 500 x g and then the pellet was resuspended in 10 ml of control medium. One milliliter of each washed sperm sample was mixed with an equal volume of either isthmic or ampullary conditioned medium. All samples were incubated in 5% CO_2 : 95% air for 10 h. Aliquots were removed at 10 min., 4 h and 6 h for assessment of sperm motility and capacitation. Sperm motility was evaluated microscopically under polarized light. Capacitation status (percentage of live sperm able to undergo the acrosome reaction) was established using chondroitin sulfate to induce the acrosome reaction in capacitated sperm (8).

QUANTITATIVE AND QUALITATIVE DETERMINATION OF GLYCOSAMINOGLYCANS IN OVIDUCTAL CONDITIONED MEDIUM

Glycosaminoglycan quantity in isthmic and ampullary conditioned media was determined as uronic acid content, by the method with Alcian Blue (9), (10).

In order to establish if the glycosaminoglycans in the isthmic and ampullary conditioned media are of chondroitin sulfate-type, we have treated the samples of conditioned medium with chondroitinase ABC (Sigma) (2 units in 1 ml Tris-HCl buffer, pH 8.0, 30 min., at 37°C). Then, we have incubated samples of sperm with these enzyme-treated conditioned media and we have tested the induction of the acrosomal reaction.

RESULTS AND DISCUSSION

The protein concentration in isthmic and ampullary conditioned media is shown in table 1.

These results were a first proof of the secretory capacity of the oviductal explants *in vitro*.

Table 1

Protein and glycosaminoglycan concentrations in oviductal conditioned medium

Oviduct region	Protein (mg/ml) (Lowry)	Uronic acid (μg/ml) (Alcian Blue)
Isthmic conditioned medium	7.6	153.73
Ampullary conditioned medium	7.2	144.44

After incubating the sperm samples with either isthmic or ampullary conditioned medium, we have determined the maintenance of sperm motility at 10 min., 4 h and 6 h. The results of this experiment are presented in figure 1.

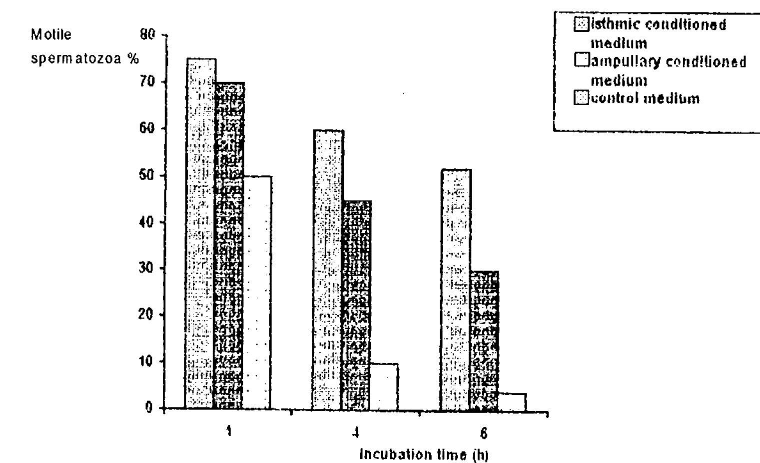

Fig. 1. — Effect of oviductal conditioned medium on sperm motility.

The experiment demonstrated that the isthmic conditioned medium was able to maintain the motility of 50% spermatozoa after 6 h of incubation, while in the control medium only 5% of spermatozoa were mobile after the same period of time. This observation may have a special significance because spermatozoa *in vivo* are considered to reside in the isthmus for up to 18 h before fertilization (11).

The sperm incubated in the presence of isthmic, ampullary or control medium was tested for the capacitation status. Chondroitin sulfate (100 μg/ml) was added in each sample after 10 min., 4 h and 6 h of incubation in oviductal conditioned medium or in control medium and then, the incubation was continued for 4 h, at 37°C. The capacitation status was established as percentage of live spermatozoa which were able to undergo the acrosome reaction (Figure 2).

The higher percentage of spermatozoa able to undergo the acrosome reaction was found in the sample with isthmic conditioned medium after 6 h of incubation. This result supports the finding of Parrish (7) and Anderson (12) showing that bovine oviductal fluid capacitated a significant number of spermatozoa

in vitro, and suggests that macromolecules produced by the isthmus may play a major role in capacitation.

The glycosaminoglycan concentrations in the isthmic and ampullary conditioned media, as uronic acid content, are presented in Table 1.

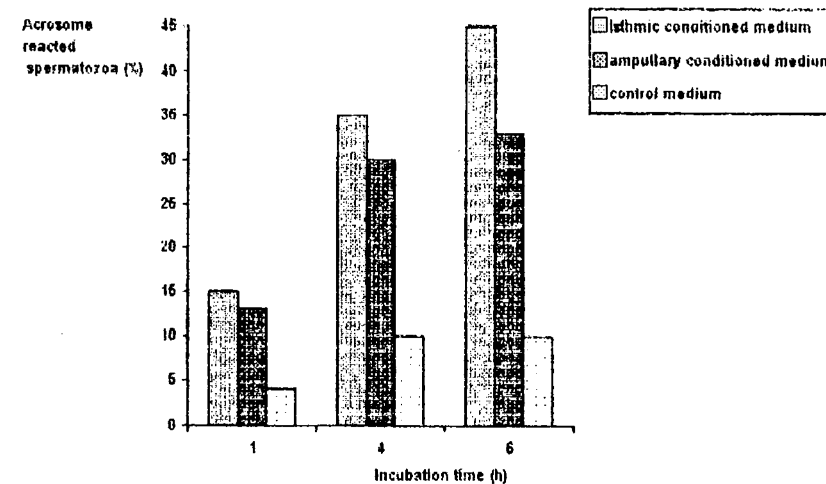

Fig. 2. — Effect of oviductal conditioned medium on capacitation status of live spermatozoa.

As it was already suggested (7), (8), glycosaminoglycans are a capacitation factor present in the oviductal fluid at the time of estrus. Proteoglycans may be another capacitating factor within the oviduct. Proteoglycans consist of glycosaminoglycans covalently bound to a core protein. The increased capacitating ability of isthmic conditioned medium may be due to an increased relative concentration of GAG covalently bound to the proteins.

We have treated the conditioned oviductal medium with chondroitinase ABC and then we incubated the samples of sperm with this enzyme-treated medium and with control medium. After addition of chondroitin sulfate, incubation and staining for the acrosomal reaction, we observed that spermatozoa incubated in the enzyme-treated conditioned medium did not undergo the acrosomal reaction, that means they were not capacitated.

We concluded that the oviduct explants secreted glycosaminoglycans of chondroitin sulfate-type *in vitro,* which are responsible for the sperm capacitation. We also observed that only capacitated sperm undergoes acrosomal reaction. These results are in concordance with our previous observations about the role of chondroitin sulfate in the induction of acrosome reaction *in vitro* (13).

Therefore, both isthmic and ampullary regions of the sow oviduct secreted proteins and glycosaminoglycans in the culture medium, but isthmic conditioned medium was more efficient in the maintenance of motility and in sperm

capacitation than ampullary conditioned medium. The glycosaminoglycans secreted in the conditioned medium are of chondroitin sulfate-type and are responsible for the sperm capacitation and acrosome reaction induction.

Acknowledgement. The authors thank Mrs. Aurelia Mihăilă for her excellent technical assistance to oviductal explant cultures.

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HISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY OF GLYCOSAMINOGLYCANS IN SOW OVIDUCT

MARIA CALOIANU*, ANCA OANCEA*, OTILIA ZĂRNESCU**

The aim of the present study was the identification, by specific methods, of glycosaminoglycan types from sow reproductive tract. Three segments of sow reproductive tract were investigated: ampulla, isthmus, and the oviduct-uterus junction. Tissue fragments from these segments were processed for light microscopy and examined after the application of the following specific methods: Alcian Blue staining at a certain $MgCl_2$ concentration, specific-enzyme incubation (chondroitinase AC, chondroitinase B, and hyaluronidase), immunocytolocalization using mouse monoclonal antibodies against chondroitin sulfate. Our results demonstrated that there are considerable amounts of glycosaminoglycans in the structure of the connective tissue of sow oviduct and they are of chondroitin 4-sulfate- and chondroitin 6-sulfate- type, and also (small amounts) of dermatan sulfate and hyaluronic acid.

The presence of glycosaminoglycans in the mammalian female reproductive tract and in its secretions was already established by biochemical methods, and by quantitative determinations in tissue extracts (1), (2).

Due to the importance of oviductal glycosaminoglycans in the fertilization process, particularly in sperm transport regulation (3), (4), (5), in ovum-spermatozoon interaction (6), (7), in embryo transport towards the uterus (8), thorough studies of the type and distribution of these macromolecules in the mentioned tissue are necessary.

Some histochemical methods for detection of acid glycoconjugates by light microscopy are used: Alcian Blue at pH 1.0 or 2.5 (9), high- or low-iron diamine (10), aldehyde fuchsin (11), dialyzed iron ferricyanide (12), Azure A (10), and Cuprolinic blue (13).

However, these methods allow histochemical evidentiation of all acid glycoconjugates in a tissue.

To identify only the glycosaminoglycans and to determine their type, we have used specific and efficient histochemical and immunohistochemical methods such as the specific-enzyme digestion and the immunocytolocalization with monoclonal antibodies.

MATERIALS AND METHODS

TISSUE PREPARATION

The studied female reproductive tract segments were the ampulla, the isthmus, and the oviduct-uterus junction. These tissue segments were collected from sows before estrus (the hormonal stage was established by examining the ovaries, depending on the size of ovarian follicles).

The analyzed segments were fixed and processed for light microscopy.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 42, N° 1, P. 63–71, BUCAREST, 1997

HISTOCHEMICAL METHODS

All procedures were carried out so that each batch of sections (control and experimental series) were stained in the same solution at the same time.

All staining procedures were conducted in parallel, some sections of each group of serial sections being digested with specific enzymes before staining.

We have used the following procedures:

— Staining with Alcian Blue (0.05% in 0.2 M acetate buffer, pH 5.8) containing 0.06 M magnesium chloride (9).

— Staining with Alcian Blue (0.05% in 0.2 M acetate buffer, pH 5.8) containing 0.5 M magnesium chloride (9).

— Digestion experiments with specific enzymes:

— chondroitinase AC (Sigma) (2 units/ml) in 0.05 M Tris-HCl buffer, pH 7.3;

— chondroitinase B (Sigma) (2 units/ml) in 0.05 M Tris-HCl buffer, pH 7.5;

— testicular hyaluronidase (Choay Laboratory) (1 000 UI/ml) in 0.1 M phosphate buffer, pH 6.7.

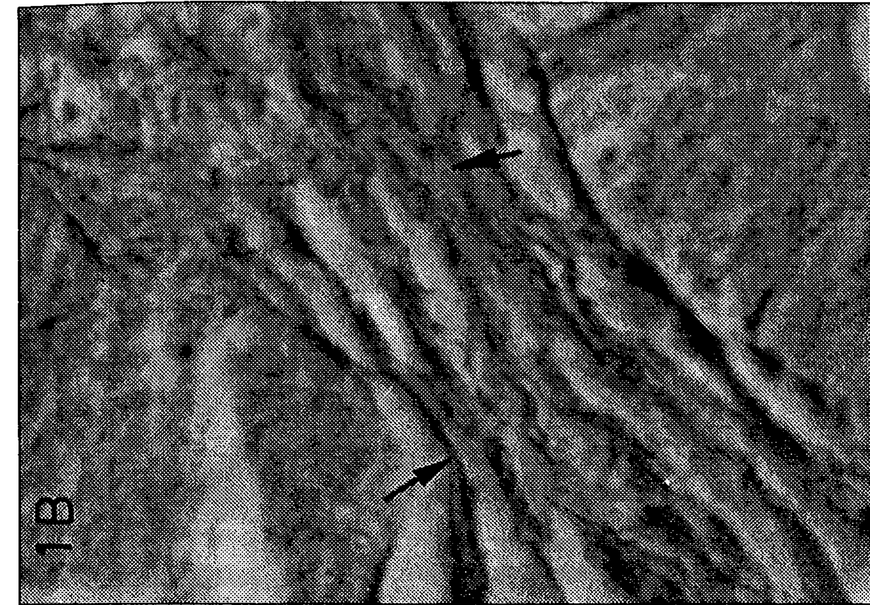
The slides were incubated in the presence of enzymes or in buffer (control) for 3 h, at 37°C and then were stained with Alcian Blue containing 0.5 M $MgCl_2$.

— Immunocytolocalization using monoclonal antibodies: tissue sections were incubated in the presence of the primary antibody (mouse antibody against chondroitin sulfate) overnight at 4°C, washed in PBS and then incubated in the presence of the secondary antibody (goat antibody against mouse IgG) coupled with peroxidase, for 1 h, at room temperature. The sections were washed in PBS, incubated in the presence of 3,3′ diaminobenzidine and H_2O_2 and contrasted with 0.1% nuclear red.

RESULTS AND DISCUSSION

When tissue sections from sow reproductive tract were stained with Alcian Blue in the presence of 0.06 M $MgCl_2$ (using nuclear red as counterstain) at pH 5.8, all acid glycoproteins stained blue (Fig. 1 A-D). This method allows us to observe the high quantity of acid glycoproteins in all tested segments, at the level of epithelium or in the extracellular matrix, but it is, however, an inadequate method to distinguish the type of these glycoproteins in tissues such as primate oviduct (14).

After staining the tissue sections with Alcian Blue in the presence of 0.5 M $MgCl_2$, only strongly sulfated glycoproteins stained blue. Therefore, this method allows us to observe the presence and the distribution of glycosaminoglycans



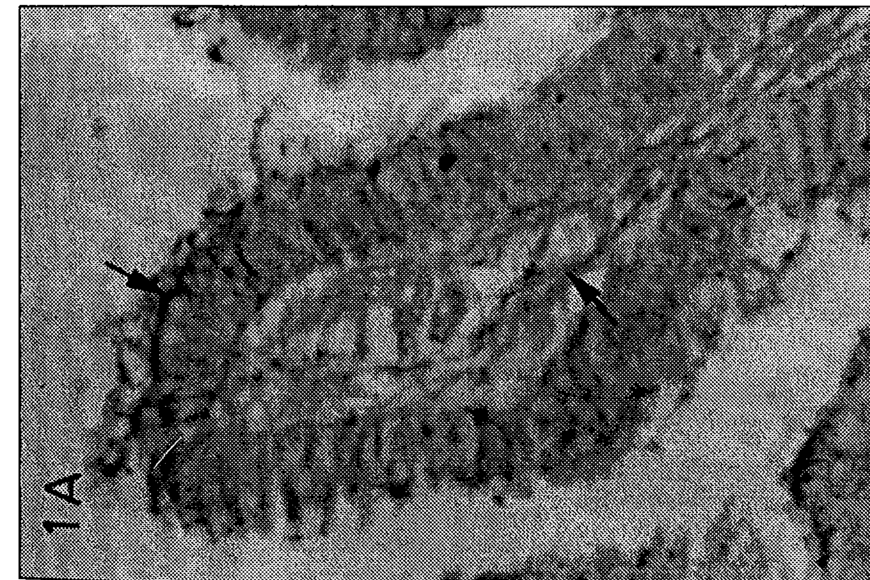


Fig. 1. — Sow reproductive tract segments stained with Alcian Blue in the presence of 0.06 M $MgCl_2$ (counterstain with nuclear red).
All acid glycoproteins stain blue (arrows).
A — ampulla (× 320)
B — isthmus (× 640)

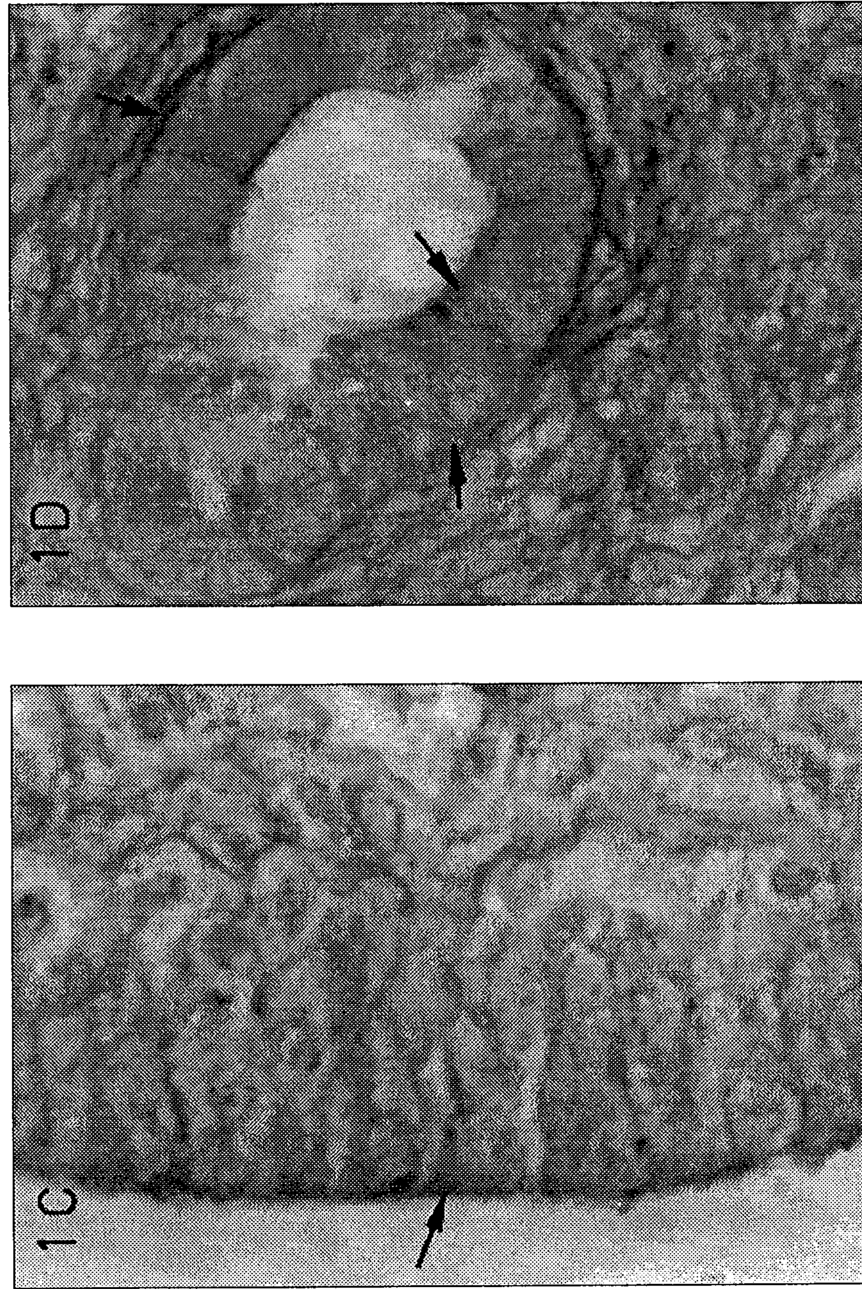
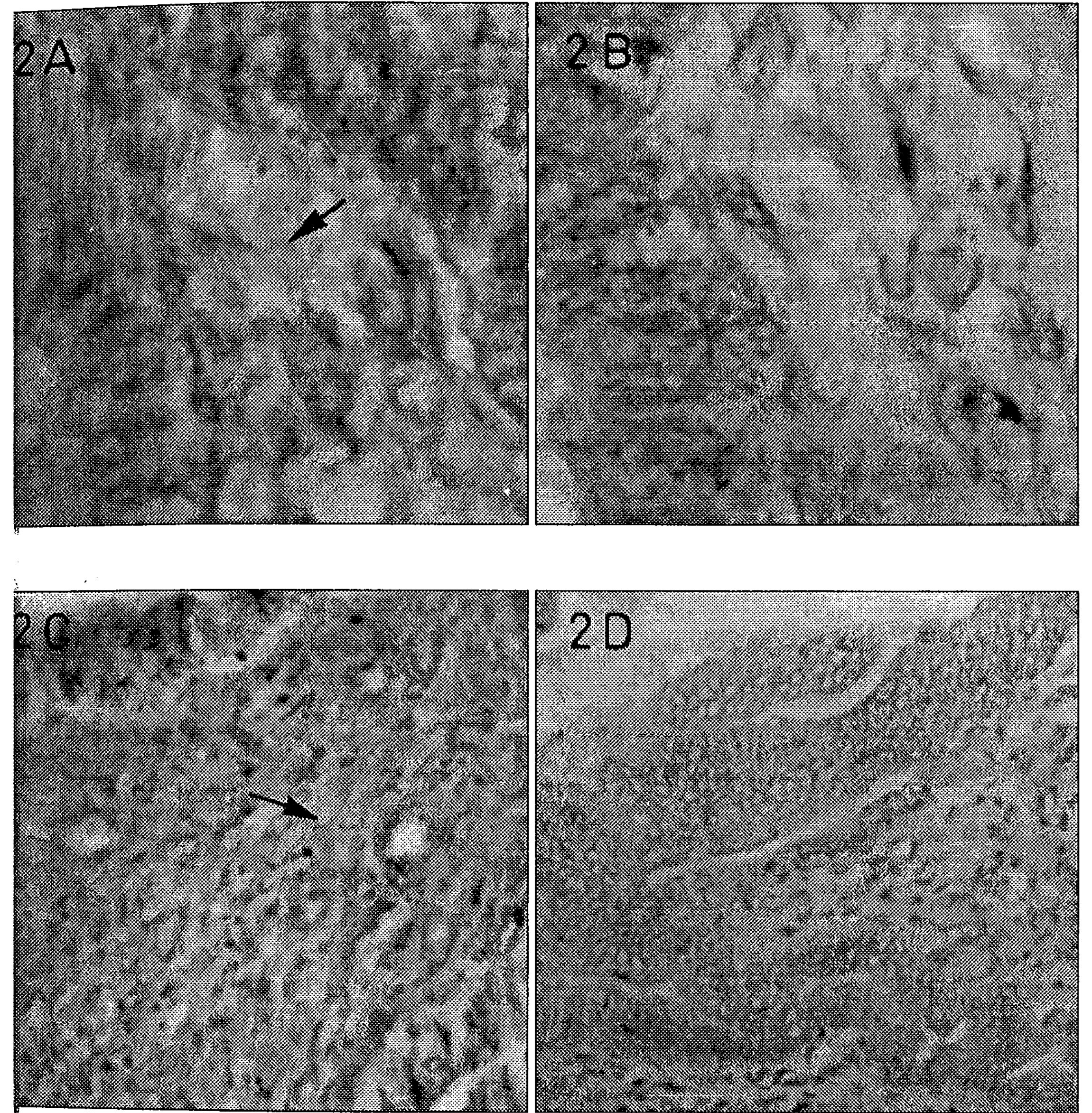


Fig. 1. — Sow reproductive tract segments stained with Alcian Blue in the presence of 0.06 M $MgCl_2$ (counterstain with nuclear red).
All acid glycoproteins stain blue (arrows).
C — oviduct-uterus junction (epithelium) (× 640)
D — uterine cervix (× 320)



2. — Sow reproductive tract segments stained with Alcian Blue in the presence of 0.5 M $MgCl_2$. Strong sulfated glyco-teins stain blue (arrows) (controls). The same reproductive tract segments were incubated in the presence of chondroitinase AC before staining with Alcian Blue.

A — ampulla — control
B — ampulla — after chondroitinase AC digestion
C — isthmus — control
D — isthmus — after chondroitinase AC digestion

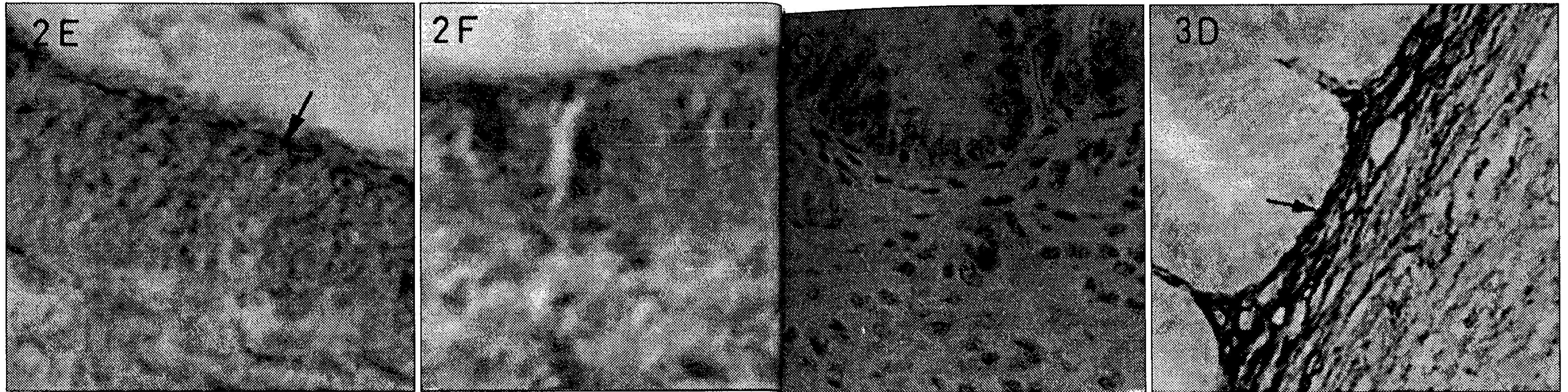


Fig. 2. — Sow reproductive tract segments stained with Alcian Blue in the presence of 0.5 M $MgCl_2$. Strong su glycoproteins stain blue (arrows) (controls). The same reproductive tract segments were incubated in the prese chondroitinase AC before staining with Alcian Blue.
E — oviduct — uterus junction — control
F — oviduct — uterus junction — after chondroitinase AC digestion

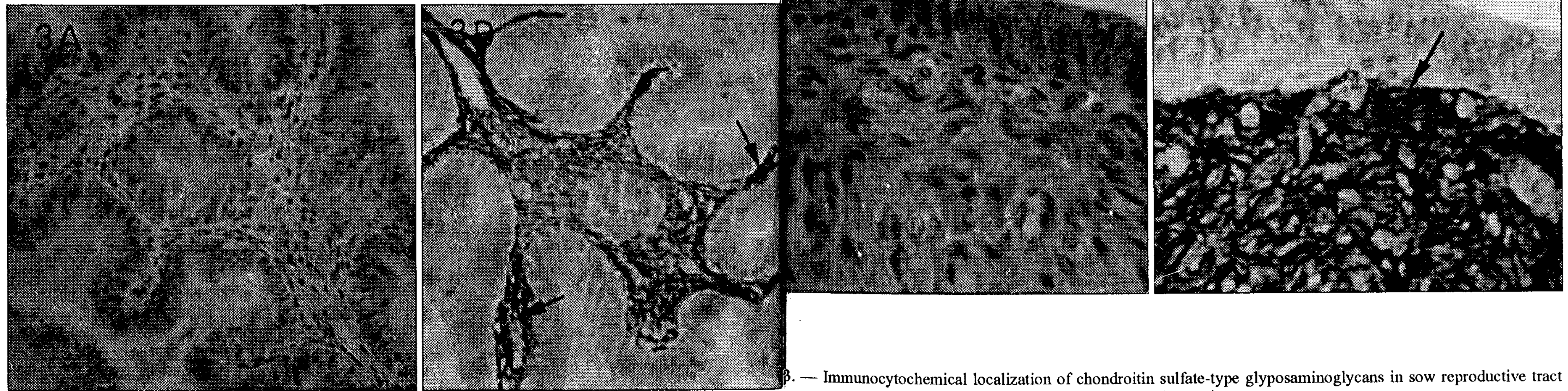


Fig. 3. — Immunocytochemical localization of chondroitin sulfate-type glycosaminoglycans in sow reproductive segments (arrows)
A — ampulla — control
B — ampulla — after immunocytochemical reaction

3. — Immunocytochemical localization of chondroitin sulfate-type glyposaminoglycans in sow reproductive tract segments (arrows)
C — isthmus — control
D — isthmus — after immunocytochemical reaction
E — oviduct — uterus junction — control
F — oviduct — uterus junction — after immunocytochemical reaction

(which are strongly sulfated glycoproteins) in the sow reproductive tract. Incubating samples of tissue in the presence of specific enzymes before staining with Alcian Blue in the presence of 0.5 M $MgCl_2$, we can identify the type of these glycosaminoglycans in the studied segments of reproductive tract.

In the ampulla of the sow oviduct, after staining the tissue sections with Alcian Blue in the presence of 0.5 M $MgCl_2$, we have observed the localization of sulfated glycosaminoglycans (stained blue) both in the chorion from the mucous membrane fold axis and at the folds basis (Fig. 2 A).

In the isthmus of the oviduct, the positive reaction (blue staining) is present at the level of mucous membrane chorion and at the level of connective tissue surrounding the blood vessels (Fig. 2 C).

In the region of oviduct-uterus junction, we have observed a strong positive reaction at the level of connective tissue beneath mucosal epithelium (Fig. 2 E).

Our results concerning the presence of sulfated glycoproteins at different levels of sow reproductive tract are in concordance with the observations, obtained by other staining methods, concerning the distribution of these macromolecules in the rabbit oviduct at estrus (15).

Incubating the tissue sections in the presence of chondroitinase AC before staining them with Alcian Blue, the structures stained in blue in the figures 2 A, 2 C and 2 E (that are control samples), were not stained anymore (Fig. 2 B, 2 D, 2 F). That means that the sulfated glycoproteins present in the structure of sow reproductive tract are glycosaminoglycans of chondroitin 4-sulfate and chondroitin 6-sulfate type. We have also used chondroitinase B for incubating the tissue sections and, after staining these samples with Alcian Blue, we have observed a very weak blue stain (data not shown); therefore, in the studied tissue there is also a little quantity of chondroitin sulfate B (dermatan sulfate).

The results concerning the localization of glycosaminoglycans in sow reproductive tract obtained by the described methods are in concordance with those obtained by using another specific method for glycosaminoglycans — the Ruthenium Red staining (16).

We have also incubated tissue sections from all the studied segments in the presence of testicular hyaluronidase (17) and, after staining by the Alcian Blue — neutral red method, we have observed that the structures stained blue in control samples were unstained (data not shown). Knowing that hyaluronic acid, chondroitin sulfate A and chondroitin sulfate C remain unstained after hyaluronidase treatment (18), we can conclude that these are the types of glycosaminoglycans found in the structure of the studied tissue.

The presence and localization of glycosaminoglycans in oviductal tissue were also investigated by using an immunocytochemical procedure.

The results of this procedure performed at light microscopic level have indicated that, in the ampulla, the immunocytochemical reaction appeared positive, particularly in lamina propria of the mucosa (Fig. 3 A, B).

In the isthmic region of the oviduct it was evidentiated a larger quantity of chondroitin sulfate than in the ampulla, by the strong immunocytochemical reaction (Fig. 3 C, D).

In the oviduct-uterus junction, the intense immunocytochemical reaction at mucosal level revealed plenty of chondroitin sulfate in the connective tissue between glands (Fig. 3 E, F).

The immunocytochemical studies were in concordance with the above histochemical observations and specified the presence of chondroitin sulfate-type glycosaminoglycans in the sow reproductive tract segments, clearing the more general localization of various glycoproteins reported in the cow oviduct (19) at estrus, by the peroxidase-antiperoxidase immunocytochemical method.

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A STATISTICAL ANALYSIS ON THE *DROSOPHILA MELANOGASTER* EYE ELECTRORETINOGRAM

M. ISAC*, D. E. CREANGĂ**, R. M. ISAC*

Abstract. In this paper we focused on the bioelectrogenesis at the level of the compound eye photoreceptor cells, revealed by the action potential induced by the light induced in the cells microvillar membranes. We present the results of our experimental studies concerning the electrophysiological investigation of the compound eye global electrical activity (electroretinogram). The biological material was the fly *Drosophila melanogaster* cultivated by us following special prescriptions and the electroretinogram characteristics that we obtained are in good agreement with the literature reports. A statistical analysis of the experimental signal time dependence was carried out using the rapid Fourier transform, the probability distribution and the polynomial fitting.
Key words: electroretinogram, *Drosophila melanogaster,* statistical analysis.

INTRODUCTION

Though the invertebrates are situated on an inferior position in the species evolution scale, however in some cases they present very developed organs both from the anatomical and physiological points of view, like the eyes in some arthropods. Our interest being focused on the composed eye of the *Drosophila melanogaster* it is important to mention, in the first place, its large spectral sensitivity both in the visible and in the ultraviolet range as well as its capacity of responding to polarized light.

Each of the about 700 ommatidia or simple eyes present in every of the two compound eyes of this fly is formed by six peripheral visual cells (Fig. 1), having connections with the first order ganglionaris, *lamina* and two central, overlapped visual cells, having connections at the level of the second order ganglionaris, *medulla.*

Microspectrophotometric, electrophysiological and behavioral studies carried out on the compound eye of *Drosophila melanogaster* revealed that the central photoreceptors R 1–6 are characterized by two spectral sensitivity maxima, one in the ultraviolet range, at $\lambda = 350$ nm and the other in the visible range, at $\lambda = 470$ nm. The cells R7 have a spectral sensitivity maximum at $\lambda = 370$ nm, in ultraviolet, while the R8 cells, situated R7, have the visible sensitivity maximum at $\lambda = 490$ nm, as a consequence of the screening action of R7 [1]. The behavioural studies carried out by H. Ch. Spatz and C. Hernandez de Salomon [2] in order to clarify the global spectral sensitivity of the *Drosophila melanogaster* compound eye, showed that in the range 406–525 nm the spectral sensitivity is almost the same but it rapidly decays to zero when λ is greater than 600 nm.

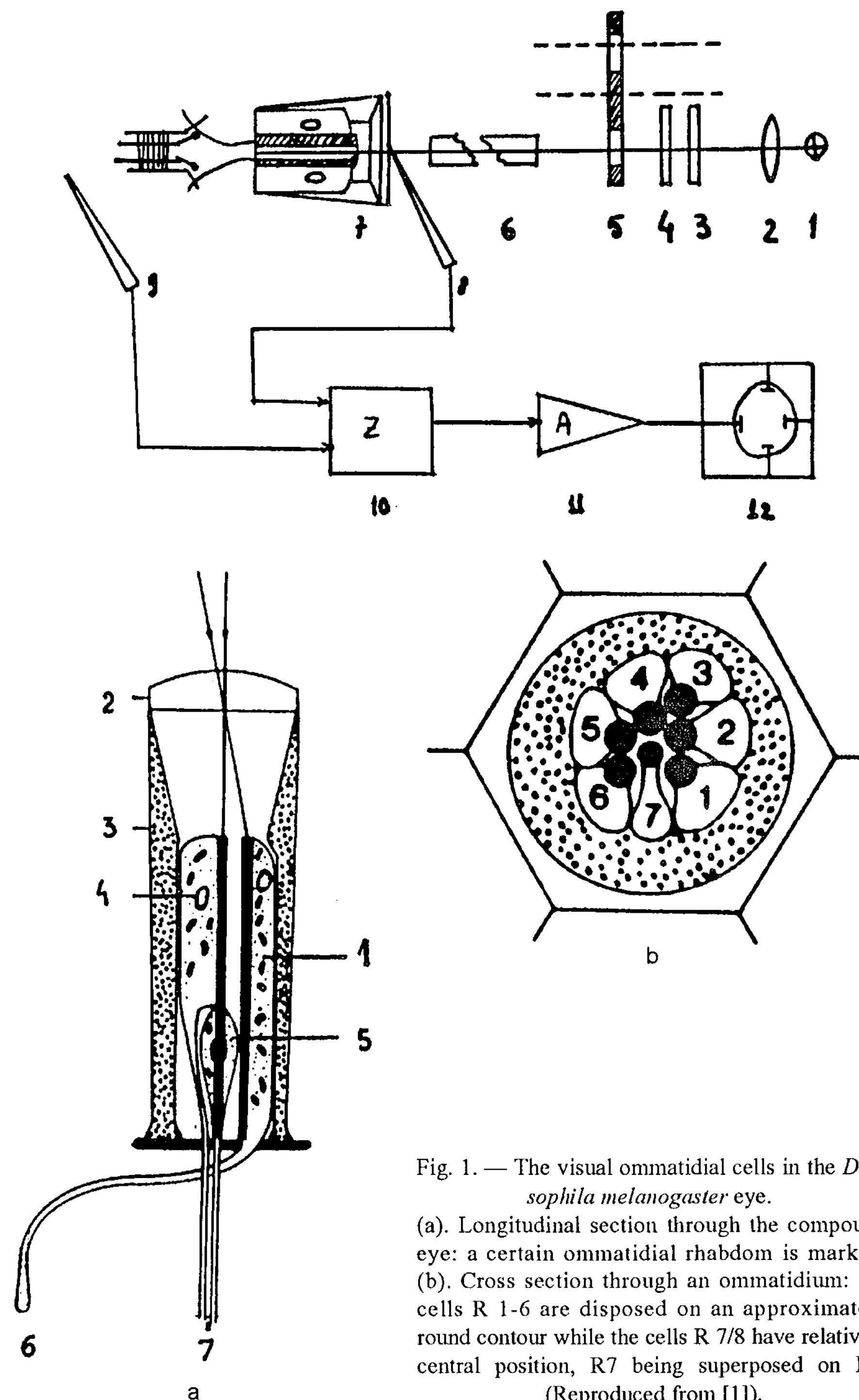


Fig. 1. — The visual ommatidial cells in the *Drosophila melanogaster* eye.
(a). Longitudinal section through the compound eye: a certain ommatidial rhabdom is marked.
(b). Cross section through an ommatidium: the cells R 1-6 are disposed on an approximately round contour while the cells R 7/8 have relatively central position, R7 being superposed on R8. (Reproduced from [1]).

H. Ch. Spatz, T. Speck, M. Mutz and S. Ohl [3] used electroretinographic methods to point out the role of the central visual cells in comparison to the role of the other photoreceptors in the eye. They showed that the central visual cells are the main responsibles for the appearance of the principal component of the electro-

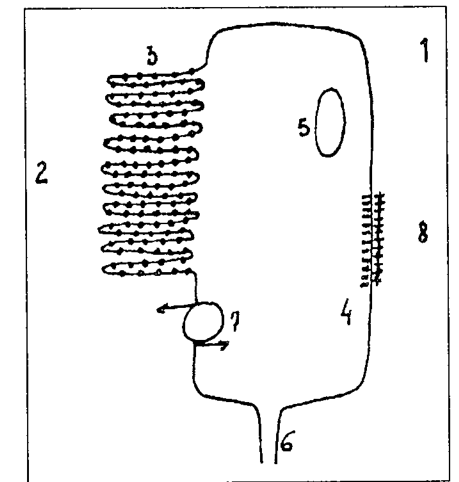


Fig. 2. — The components of the electroretinographic response obtained for a flickering light excitation, the stimuli frequency being of about 20 cycles per second.

retinographic signal, the *receptor potential*, (a significant amplitude and a large duration of time) (Fig. 2) the other components being: *the pre-potential* (a relatively small amplitude component), the transient component *lamina-on transient*, (a rapid signal but with a significant amplitude), and the *post-potential* (the larger duration component). Other studies using *Drosophila melanogaster* mutants, characterized by some deficiences at the level of their photoreceptor cells, confirmed the important role played by the R7 cells in the electroretinogram (ERG) receptor potential component appearance as well as the significant contribution of the *lamina ganglionaris* cells to the appearance of the component *lamina on transient* [3]. All these basic results in the investigation of the *Drosophila melanogaster* eyes supplied the researchers in the field of the compared physiology but also in genetics and biophysics, with very important data concerning the structure and functioning of the diptera visual analyzer.

In the microvillar structure of diptera visual cell, named rhabdomer, at the level of the cell membrane (Fig. 3) a significant concentration of photosensitive visual pigments molecules was revealed [4]. The action of light on the photosensitive pigment molecules involves o cascade of biochemical modifications and then, by still incompletely clarified mechanisms, some permeability modifications

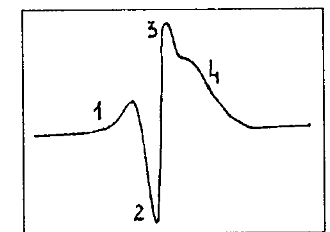


Fig. 3. — The compound eye visual cell structure. 1 — the visual cell, 2 — the rhabdomer, 3 — microvills, 4 — cell membrane, 5 — nucleus, 6 — axon, 7 — ionic pomp Na^+-K^+-ATP-ase, 8 — electrical double layer.

in the visual cells membranes occur, so that the intra- and extra-cellular concentrations of ions (Na^+, K^+, Ca^{2+}, Cl,...) change too. The electrical charge and the electrical potential are also modified both at the internal and the external membrane faces. The action potential which is generated consists mainly in a depolarization potential. The recordings of the intracellular electrical potential variations can give the most intrinsic image of the bioelectrogenesis in the compound eye photoreceptors. But, as a consequence of the fact that all the biological tissues are good electrical conductors, this electrical potential variation induced by the light action can propagate until the eye surface, where the electroretinographic recordings are able to emphasize it as well without the eye penetration. This type of measurement was also our experimental study method.

MATERIALS AND METHODS

In order to record the fly eye response to the light action we used the device already constructed in the Biophysics Laboratory of the Biology Faculty (University "Al. I. Cuza", Iași) [5]. The principal parts of this set-up are: the lighting system, the eye electrical response reception system and the system for visualization and measurement (Fig. 4).

1. The light source	7. Compound eye
2. Condenser lens	8. Measurement microelectrode
3. Thermal protection filters	9. Reference microelectrode
4. Neutral filters	10. Impedance adapter
5. Slides disc	11. Amplifier
6. Glass fiber wave guide	12. Oscilloscope

Fig. 4. — The experimental set-up for electroretinographic recordings.

The lighting system is composed by an incandescence lamp, a convergent lens assemble able to concentrate the light flow, a wave guide made of glass fiber, an adjustable diaphragm, an absorbent disc with transparent slights rotated by a little motor with adjustable and measurable speed (for the flickering light delivering), a set of neutral filters to control the light intensity and an infrared filters set (for the eye thermal protection).

The electrical response reception system is composed mainly by a couple of electrodes that are actually two glass fine micropipettes having a cross section diameter of several microns at the contact with the eye; the micropipettes are filled with a Ringer type solution and two fine wires of Ag-AgCl are immersed in there. One of these two electrodes, the measure electrode is brought in contact with the eye surface while the other, the neutral electrode is fixed in the thorax. The electrical signals are captured between the two electrodes and then lead to the visualization and measurement system.

The visualization and measurement system is composed by an impedance adapter and a cathodic oscilloscope, on the screen of which the electrical responses induced in the fly eye by the light stimuli can be seen and analyzed. In the front of the screen, a photographic device is placed to record the experimental results.

As auxiliary parts we used an electrical warmed wire, suitable to fix the flies in paraffin, a special vessel for the flies treatment with ether, a couple of microscopes for increasing the operator ability and a projection device for the analysis of film recorded images.

We used *Drosophila melanogaster* flies, a biological material used more and more though at the beginning the electrophysiologists preferred *Musca domestica* and *Calliphora erythrocephala*. The advantages of this biological material used are given by the relatively short multiplication time (several days), the availability of the culture medium components and by the existence of mutants with different peculiarities, including phototransduction ones, very useful in comparative studies. The flies aged 3–8 days have been withdrawn from the culture tubes and passed to the special ether vessel where they are let to stay for some seconds. After the flies slipped, they are picked out and placed on a paraffin sheet, where they are fixed (working with the first microscope) so that one eye remains free and available for the measurement.

The paraffin sheet bearing the samples is transferred to the second microscope and one by one the flies are brought under the light spot and the two electrodes are also adjusted. When the flies begin to move again (the ether effect disappeared), after resting for some minutes in the dark, the experiment may start.

EXPERIMENTAL RESULTS AND INTERPRETATION

We record on the photographic film the electroretinographic signals obtained for flickering light (the stimuli frequency was of about 20 cycles per second). We worked with white light and have used the wild type strain *Drosophila melanogaster* (Algeria line, red eyed).

One can see that the signal that we obtained (Fig. 5a) is in good agreement with the literature data [2, 3].

Because the electroretinographic response has the most simple form in the case of the mutant *ebony*, where the transient components are extremely low or even missing (Fig. 5b), the analysis we performed was designed as a comparison between the wild type and the *ebony* mutant.

The recorded signals were statistically analyzed as temporal functions by means of the probability distribution, the polynomial fitting and the Fourier transform, using specialized computational programs.

In the case of the *ebony* mutant the electroretinographic response leads to a probability distribution histogram (Fig. 6a) characterized by a group of peaks having medium amplitudes in the region of large electrical response values another higher peak at the limit of the smallest values region. For the wild type

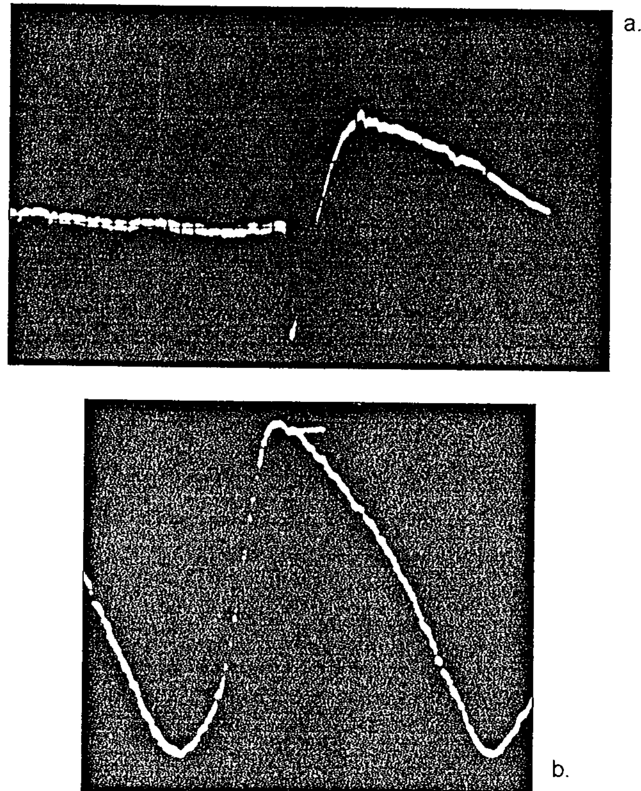


Fig. 5. — A photographic recording of the electroretinographic answer obtained on the experimental device in our laboratory using *Drosophila melanogaster* wild type and white light (a) and the ebony mutant (b). The flickering light frequency was of about 20 cycles per second.

the probability distribution histogram (Fig. 6b) supplementary contains the peaks placed at the left of the principal one and probably correspond to the *lamina-on-transient* component. Their amplitudes are of the same order of magnitude as those of the right side peaks, fact which can be associated with the observation that the amplitudes of the *receptor potential* and *lamina-on-transient* components are usually similar.

In order to get a quantitative expression for the analyzed signal we looked for the polynomial approximation, by means of the less square method of the experimental data points series. The 3rd order polynomial obtained in the case of the *ebony* mutant and the 4-th order polynomial in the case of the wild type are given respectively by the relations:

$$x(t) = 2.56 + 7.44t - 4.72t^2 + 9.94t^3 - 5.98t^4$$

$$x(t) = 1.57 + 3.00t - 6.11t^2 + 3.25t^3$$

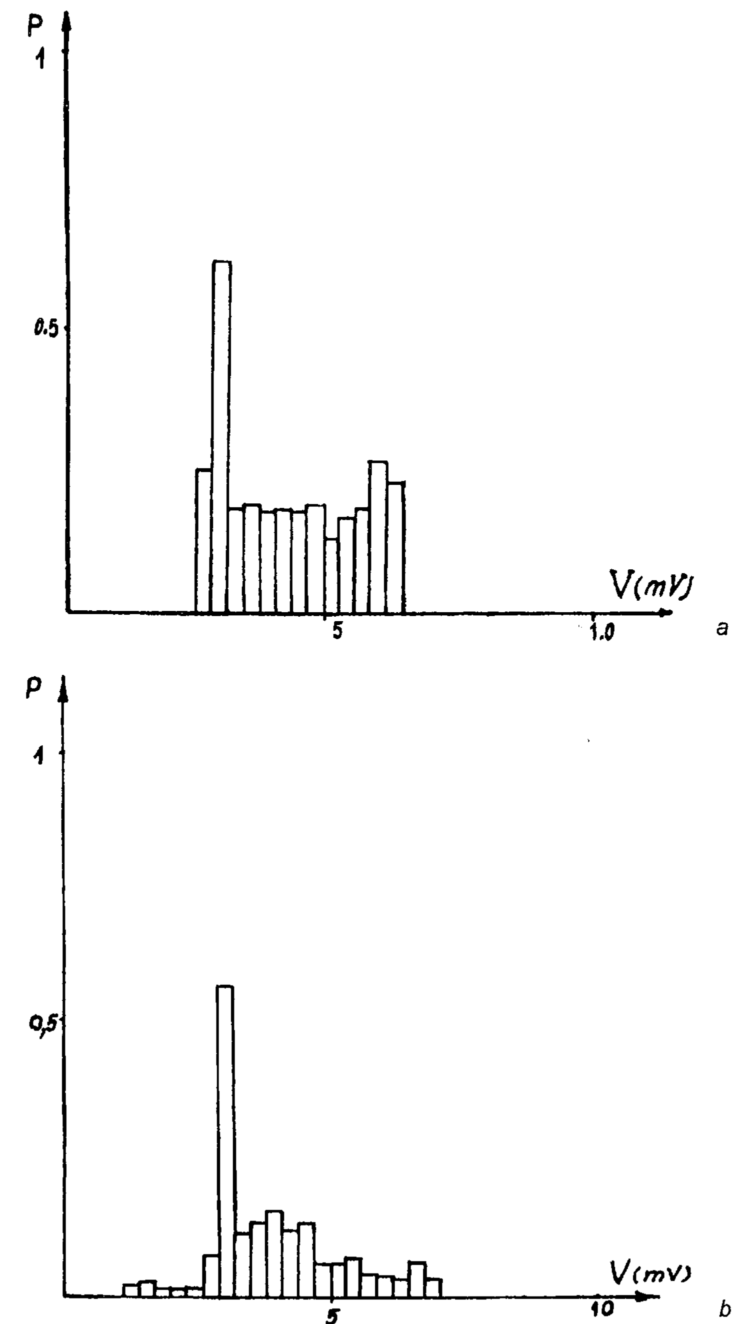


Fig. 6. — The probability distribution histogram for the temporal data points series obtained from the electroretinographic signal. (a) The wild type. (b) The ebony mutant. The frequency interval was divided into 32 equal sub-intervals.

where $x(t)$ is the membrane electrical potential and t is the time. The value of x is taken into an arbitrary reference system, convenient to our numerical analysis, so that at the moment $t = 0$ the electric potential (correspondig to the light excitation lack) is of about 2.8 mV. It means that the numerical values of the polynomial coefficients depend also on the choice of the reference system while their size, relatively to each other, depends on the eye characteristics as well on the light stimuli parameters.

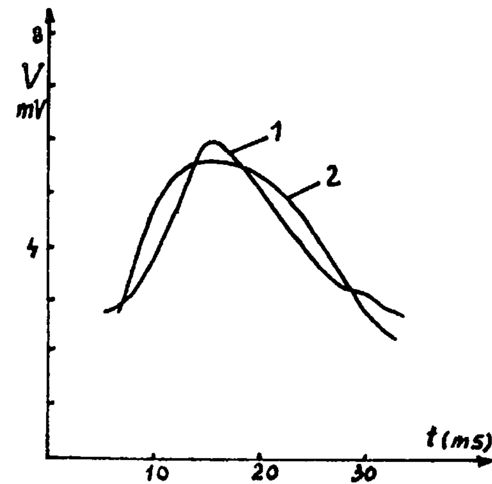


Fig. 7. — The semi-logarithmic representation of the ERG signals decomposition on the basis of a rapid Fourier transform. (a) The wild type. (b) The ebony mutant. The number of the frequency range subintervals is 16.

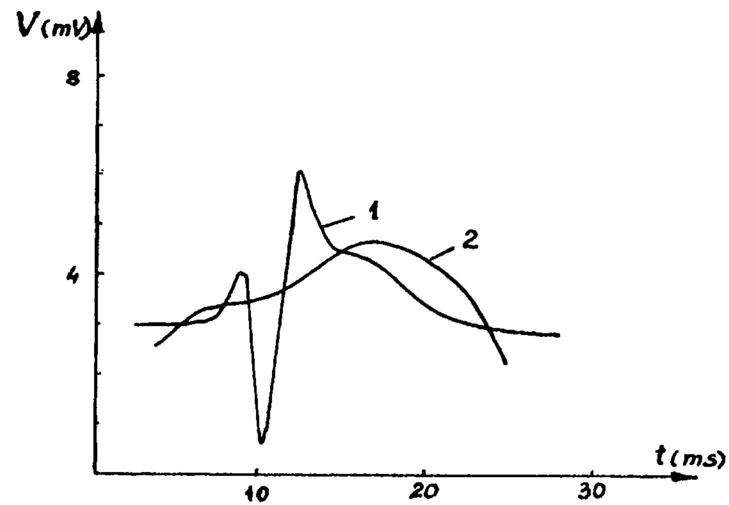
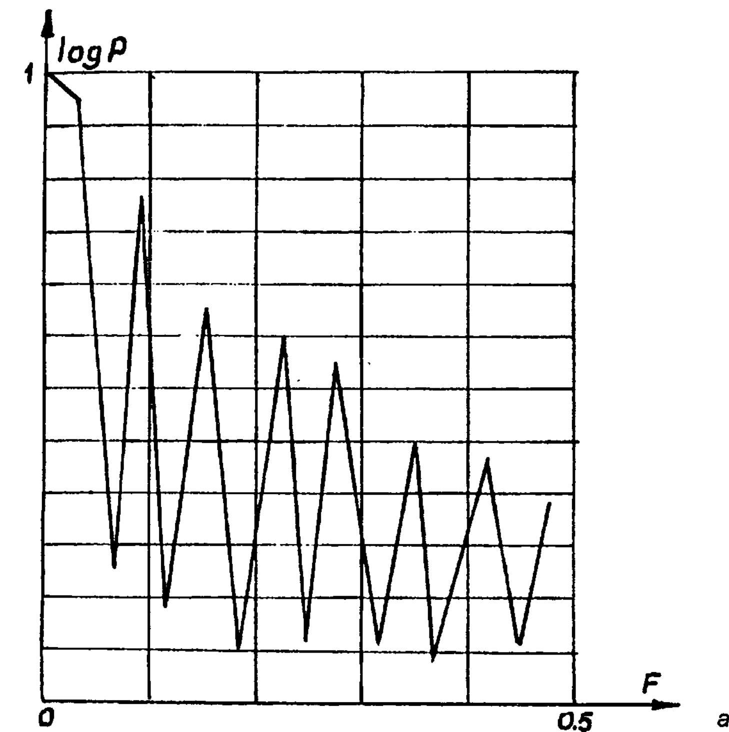


Fig. 7 b

Though the coefficients in the above relations are comparable, when plotting the two polynomials (Fig. 7 a,b), one can see that, in spite of the fact that the rapid *lamina-on-transient* component in the wild type is not "followed", the



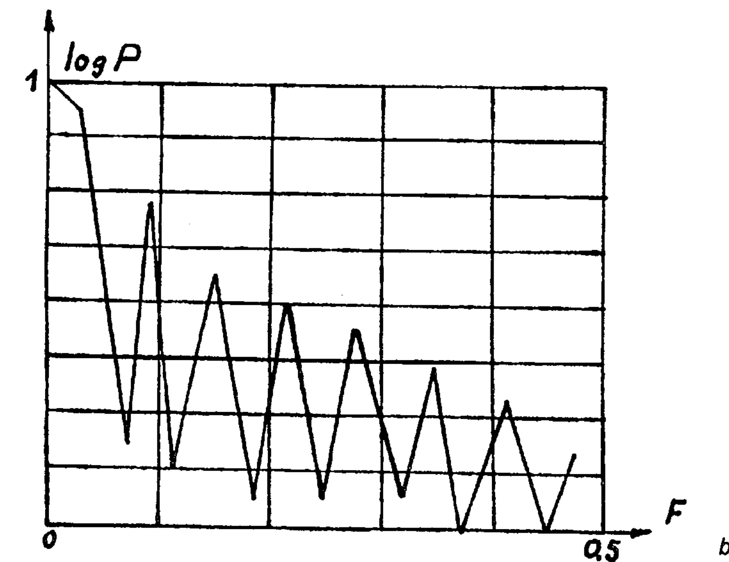


Fig. 8. — The polynomial fitting. The curve 1 corresponds to an experimental data points recording while the curve 2 corresponds to the polynomial approximation. (a) The wild type. (b) The ebony mutant.

general signal shapes are rather well reproduced. So, the polynomial fitting could be used to mathematically modeling the receptor potential together with the pre-potential and the post-potential, but for the quantitative description of the extremely rapid transient phenomena another supplementary function is required.

As a confirmation of the above results, the log-linear representation of the power spectrum (Fig. 8 a,b) suggests also that the studied signal can be decomposed into a relatively small number of components and the higher the frequency the smaller the amplitude, at the smallest frequency value being present a component having an amplitude significantly higher than all the other components.

This analysis suggests the possibility of decomposing the electroretinographic response into a small number of processes, localized in different vizual cells and quantitatively expressed by polynomial functions with only 3–4 terms as well as by means of another function, yet not defined by us, able to describe the transient component.

CONCLUSION

The experimental data obtained using the special device assembled in our laboratories are in good agreement with the results reported in the literature. Working with a flickering white light we recorded the electroretinographic responses for the wild type and for the mutant ebony with the aim of obtaining a mathematical expression of the electric potential as a function of time.

The statistical analysis carried out by us suggests the possibility of a mathematical modeling, based mainly on a polynomial approximation, corresponding to the simultaneous superposition of some bioelectrogenesis phenomena in the *Drosophila melanogaster* eye.

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IONIC TISSULAR CONTENT OF SOME MOLLUSCA SPECIES FROM THE ROMANIAN SHORE OF THE BLACK SEA

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Abstract. The content of Na^+, K^+ and Ca^{2+} ions was determined flamphotometrically from the hemolymph and also from tissues of the adductor muscle, mantle and gills of mussels *(Mytilus galloprovincialis* Lmk.), in a region with polluted water and in another one, with unpolluted marine water, and — finally — from the hemolymph and foot tissue of the *Rapana venosa* Val. gasteropode in the unpolluted regions. With both species, it was observed that, in the hemolymph, the ions show much higher values than in the sea water, regardless of the water quality while, in tissues, Na^+ takes lower values and K^+ and Ca^{2+} higher values than in marine water. In the case of *Rapana,* Na^+ is lower, comparatively with marine water, both in the hemolymph and in the foot, while K^+ and Ca^{2+} are seen as higher, especially in the foot tissue. The polluted water induces specific modifications of the distribution and ions ratios in various tissues of mussels.
Key words: marine mollusca, tissular ions, pollution

Several investigations performed evidenced a series of functional modifications in marine organisms as a result of water pollution and of the action of other environmental factors [3–7]. Thus, in a previous study [6], we evidenced some characteristic modifications of the transmembranary flows of water in the case of mussels, under the influence of their living medium's pollution.

The present paper follows the dynamics of Na^+, K^+ and Ca^{2+} ions from various tissues, on the same groups of animals [6] — a reference one, from normal marine water, and another taken over from the Agigea — Constanţa Harbour, with polluted water.

MATERIAL AND METHODS

The determinations were made on tissues of *Mytilus galloprovincialis* Lmk. and *Rapana venosa* Val. collected from the Agigea-Constanţa region.

Three groups of 5 animals each have been employed, as follows: two groups of mussels (*Mytilus galloprovincialis* Lmk.) and one of *Rapana venosa* Val., a gasteropode of oceanic origin. One group of mussels has been taken over inside the harbour, containing polluted water, with oil residues and a lot of rests of dead animals, while the reference one, collected from the clean water outside the harbour, wherefrom the *Rapana* individuals have been also obtained.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 42, N° 1, P. 83–87, BUCAREST, 1997

The content of Na^+, K^+ and Ca^{2+} ions has been determined flamphotometrically, from hemolymph (H) and tissues of adductor muscle (AM), mantle (M) and gills (G) of mussels, as well as from the hemolymph (H) and foot tissue (F) of *Rapana* and, also, from the sea water of the animals'collecting stations. The values obtained have been expressed in g/100 g fresh tissue and g/100 ml water.

The salinity (S), oxygen (O_2) and organic substance (OS) have been determined by adequate methods [1, 8].

The statistic calculation of data was based on Student's test.

RESULTS

The ionic content of tissues differ as to the ion nature, type of tissue and species considered for analysis, being also different from that of marine water.

Also an obvious influence of marine water pollution upon the dynamics of tissular ions of mussels from the polluted regions, comparatively with those of the reference batch, from the non-polluted water, was observed.

The water from the polluted region (station 1) is characterised by a low oxygen concentration (O_2 = 5.30 mg/l), presence of oil residues and of lots of dead mussels, high content of organic substance (OS = 10.70 mg O_2/l), salinity (S) of 17.60 g/l and pH = 8. In the region with unpolluted water (station 2) O_2 = 8.30 mg/l, OS = 2.60 mg O_2/l, S = 18.10 g/l and pH = 8.

The ionic content in the tissues of mussels from the polluted region varies from that of the reference animals, (100%), the values being characteristic of each tissue, as follows:

H:	Na^+ = 120.79%,	K^+ = 99.29%,	Ca^{2+} = 117.23%
AM:	Na^+ = 104.72%,	K^+ = 115.95%,	Ca^{2+} = 100.02%
M:	Na^+ = 84.72%,	K^+ = 94.99%,	Ca^{2+} = 73.56%
G:	Na^+ = 89.03%,	K^+ = 95.99%,	Ca^{2+} = 61.46%

The values recorded with the reference group (station 2) have been (expressed as mg/100 g fresh tissue ± ES):

H:	Na^+ = 283.62 ± 27.68;	K^+ = 22.29 ± 3.61;	Ca^{2+} = 28.90 ± 3.44
AM:	Na^+ = 244.82 ± 5.74;	K^+ = 60.01 ± 10.09;	Ca^{2+} = 47.05 ± 1.71
M:	Na^+ = 112.97 ± 23.90;	K^+ = 203.26 ± 23.11;	Ca^{2+} = 27.58 ± 3.80
G:	Na^+ = 210.23 ± 20.83;	K^+ = 175.17 ± 6.57;	Ca^{2+} = 49.98 ± 11.37

For *Rapana venosa* Val. the values recorded (mg/100 g fresh tissue) have been the following:

H:	Na^+ = 231.59 ± 13.55;	K^+ = 19.41 ± 1.07;	Ca^{2+} = 35.92 ± 3.92
F:	Na^+ = 126.05 ± 12.13;	K^+ = 233.14 ± 14.05;	Ca^{2+} = 130.66 ± 11.37

The values of ions from marine water (mg/100 ml) have been as follows:

Station 1:	Na^+ = 288.00;	K^+ = 22.00;	Ca^{2+} = 18.24
Station 2:	Na^+ = 270.00;	K^+ = 19.80;	Ca^{2+} = 15.68

DISCUSSIONS AND CONCLUSIONS

The data obtained on the two batches of mussels analyzed show that ions distribution in tissues differs as to their structural — functional characteristics. Thus, Na^+ is found in a higher proportion in the hemolymph than in the other tissues, while K^+ evidences a reverse distribution, which may be explained by the fact that Na^+ is predominant in the extracellular medium, as K^+ is in the intracellular one. Na^+ evidences the highest amount of H, followed by AM, G, and M, for both batches of animals, while K^+ is distributed in decreasing order, as follows: M, G, AM and H.

The Na^+/K^+ ratio attains the highest value in the hemolymph and the lowest in the mantle, the polluted water bringing about certain differences between the two batches, as follows: at H — 12.20 (sample), comparatively with 10.02 (control sample), AM — 3.68 and, respectively, 4.08, M — 0.50 and 0.55, G — 1.11 and 1.20.

Ca^{2+} does not evidence significant differences between tissues, for the reference sample the decreasing order being G, AM, H and M, while for the batch from polluted water AM, H, G and M.

The K^+/Ca^{2+} ratio, known as influencing the permeability of cell membrane [6], has different values in the two groups, as follows: H = 0.98 for the control sample, comparatively with 0.89 for the batch from polluted water, in AM = 1.27 and respectively 1.74, in G = 3.50 and 5.78 and in M = 7.37 and 9.51.

The sum of the three ions (Na + K + Ca) representing a component of the extra- and intracellular osmotic pressure, evidences, too, different values (mg/100 g) for the two batches of animals: in H 340.81 for the reference comparatively with 406.37 for the batch from polluted water, in M 343.81 and, respectively, 314.01, in AM 351.88 and 385.43, while, in G 435.38 and 385.93.

An important aspect may be put into evidence when comparing the ionic values of sea water from the regions of animals' taking over with the values of hemolymph, which represents an intermediary compartment between the marine environment and the tissues. All ions subjected to analysis are characterized by higher concentrations in hemolymph than in the marine water, certain differences being observed between the control and the polluted water batch (as % *versus* water): Na^+ — 105.04% for the control batch and 184.31% for the polluted one, K^+ — 142.88% and, respectively, 127.68%, Ca^{2+} — 184.31% and 185.74%.

The observation is therefore made that mussels, although osmoconformable organisms, evidence, too, some homeosmotic possibilities, granting to them a slight osmotic independence against the marine millieu [3, 4, 6, 9].

Nevertheless, such osmoregulation mechanisms are perturbed by water pollution. The modifications induced by polluted water involve both the dynamics of the ions from tissues and hemolymph and the ionic ratios, and also the extra- and intracellular distribution of water, as analyzed in a previous paper [6]. This distribution reflects modifications of hydric and ionic transmembranary flows and also of the osmoregulation mechanism [4–7, 9], the most ample modifications being recorded at hemolymph, which represents the intermediary compartment between marine water and tissues, at gills, a tissue with breathing role and at the adductor muscle that assures reclusion under unsuitable environmental conditions [3, 4].

The dynamics of ions in *Rapana venosa* Val. is quite different from that of *Mytilus* (of the reference sample), characterized by a lower content of Na^+ and K^+ and a higher Ca^{2+} content in the hemolymph, as well as much lower Na^+ values and much higher K^+ and Ca^{2+} values in the foot muscle, comparatively with the mussel adductor muscle. Also, differences are observed between the values of the ionic ratios and of the ions sum, as follows: in H: $Na^+/K^+ = 11.93$; $K^+/Ca^{2+} = 0.54$; $Na + K + Ca = 286.92$; in F: $Na^+/K^+ = 0.54$; $K^+/Ca^{2+} = 1.78$; $Na + K + Ca = 489.85$.

Comparatively with the sea water (100%) the ions from hemolymph represent: $Na^+ = 85.77\%$; $K^+ = 98.03$; $Ca^{2+} = 229.08\%$, a reduction in the Na^+ and K^+ concentration being therefore observed, along with a considerable increase of the Ca^{2+} concentration. A similar situation was observed with water tissular distribution [6], which reflects the existence of some efficient mechanisms of osmotic regulation in this oceanic organism (*R. venosa*) adapted to the conditions of the Black Sea, characterized by a much lower salinity (about 16 g/l comparatively with about 30g/l in the ocean) [4–7].

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SOME PRELIMINARY BIOMEDICAL OBSERVATIONS ON U-CONTAMINATION BY INGESTION IN RATS

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GABRIELA DUMITRESCU*, MARIA CUCU and E. I. IANCU*

A number of observations on acute body contamination by ingestion are presented as part of a broader research on the internal contamination with uranium. Red and white blood cell counts for two different diets, with and without vitamin therapy, are presented in comparison with a normal diet. Some microscopic samples from the animals' stomachs, lungs and kidneys were examined, and serious damage of the latter was reported.

International studies have revealed that fissile elements such as U, Th and Pu have a double impact on animal bodies caused by the chemical properties of the elements, one the one hand, and by their radioactive properties on the other hand.

The chemical effect consists in a high level of toxicity, while the radioactive one consists in cell and tissue damage inflicted by radiation (Assessment of U and Pu Body Burdens) (1).

For this reason, precise protection standards have been set to show the maximum admitted doses for the inhalation and ingestion of fissile materials, particularly U and Pu.

A research program has been drawn up to gather additional data on U deposition in, and elimination rate from, body organs for several contamination routes, including inhalation, ingestion, and superficial wounds. Quantitative assessments have been performed by the track detection technique used by Cucu M. and Danis A. (2–4).

The paper reports a number of preliminary observations following acute internal contamination by U ingestion. The main purposes of the investigation were:

— to observe the hematologic changes occurring in contamined subjects, compared with normal hematologic structures;

— to test the role of an additional vitamin uptake in reinforcing body resistance to U toxicity; and

— to determine structural alterations in certain vital tissues (organs) as a consequence of U irradiation.

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MATERIALS AND METHOD

Healthy, young Wistar-London male rats were used in the experiments.

As a contamination agent we employed a uranyl nitrate solution with a concentration $C_U = 5.58$ gU/l, buffered with NaOH up to a pH = 6, where 1 $gU_{natural} = 25.87 \times 10^3$ Bq.

Internal contamination occurred by ingestion, with the U solution crammed into the subject stomachs at a rate of 1 ml per day. The total activity administered was calculated to equal 2 × Annual Limit on Intake (ALI) to obtain an internal irradiation dose equivalent corresponding to 2 × H_{annual}, which is an exceptional irradiation value. (5)

The quantity of contaminant solution to provide a value 2 × H_{annual} was calculated in terms of subject mass as follows:

$$1 \text{ Bq} = 40 \ \mu\text{g} \ U_{nat}$$

$$H_{animal} = H_{human} \frac{m_{animal}}{m_{human\ standard}} \quad \text{where } m_{human\ standard} = 72 \text{ kg}$$

To attain an internal irradiation equivalent to 2 × H_{annual}, in accordance with the latest limits on internal U contamination (Annals of the ICRP, Publ. 61, 1991), we used 8 ml of U solution per animal. The initial weight (body mass) of the rodents was in the range 150–160 g. The target irradiation dose was achieved by administering a daily 1 ml of U solution for 8 days.

The control rat was kept in normal living conditions for 15 days, and developed normally increasing in weight from an initial 151 g to 159 g when sacrificed.

The animals were beheaded and the blood was collected on anticoagulant, then the red and white cell structure was analysed.

The first experimental rat received a daily 1 ml of uranyl nitrate for 5 straight days, plus half the daily food ration and water *ad libitum*. It was found dead on the sixth day.

The second experimental rat received 1 ml of uranyl nitrate per day for 5 days in a row. The treatment was suspended on the 6th and 7th days, then resumed on the 8th, 10th and 12th days. After further 7 days, the animal was sacrificed and its blood was collected for red and white cell tests. Water and food were provided *ad libitum* throughout the experiment duration.

The third experimental rat was treated with the same dose of uranyl nitrate and in the same timing conditions as the second. Water and food were again

available ad libitum, only the drinking water contained in addition a polyvitamin compound of the Electovit (Pantovit) type. Every pill contained:

Vitamins:	B_1	0.006 g
	B_2	0.006 g
	B_6	0.003 g
	B_{12}	2 gamma
	PP	0.010 g
Calcium panthotenate		0.010 g
Calcium phosphoricum		0.050 g

The sugar coating of the pills was removed, and the inner core was pounded and divided into 5 equal rations, which were administered at a rate of one per day dissolved into the drinking water ration of 20 ml. Polyvitamin started to be administered at the same time as the uranyl nitrate treatment, and continued for 12 more days after the contaminant treatment had been halted, until the animal was sacrificed. Then the blood was collected for red and white cell counts.

Microscopic samples of lung, stomach and kidney tissue were collected from the second experimental rat. For light microscopic investigations, small fragments of rat kidney were fixed in formalin 35% or Bouin, then embedded in paraffin, cut into 6–7 μm thick sections and stained with hematoxylin eosine.

RESULTS AND OBSERVATIONS

The blood cell count of the control rodent revealed:

— hemoglobin	13.5 g ‰
— leukocytes	14.000
— segmented neutrophiles	46%
— lymphocytes	52%
— plasmocytes	2%

No morphological alterations were found in any of the blood elements whatever their degree of maturation. The cells had a normal appearance, characteristic of a healthy animal.

The first two experimental animals exhibited the following behaviour characteristic in the daytime:

— crouching position
— bristly fur
— torpor
— tachypnea

The third rat showed partly bristly hair and a slight torpor. The first animal took in the food and water entirely. The second absorbed only part of the food and water ration. The third took in the ration almost entirely. Dissection of the first animal revealed a strong brain hemorrhage and multiple tumorettes on both lungs and kidneys, which appeared bleached (whitish). On the 17th day after the first uranyl nitrate ration, the second experimental rodent exhibited a paresis of the right foreleg, which compelled authors of the experiment to sacrifice it. A blood sample collected from the animal showed a blood cell count as follows:

— hemoglobin	13 g ‰
— leukocytes	3.650
— erythrocytes	bull's eye-shaped, broken and frayed
— segmented neutrophiles	very few and partly destroyed
— lymphocytes	broken and fragmented
— plasmocytes	broken
— toxic granules	present

Dissection of this second animal revealed a brain hemorrhage, 2 tumorettes on the right lung, and kidney bleaching. The third experimental animal, the one on vitamin therapy, was kept for 12 more days after the last contamination, then sacrificed when it was losing weight. The blood sample collected from the rodent showed the following contents:

— hemoglobin	13 g ‰
— leukocytes	5.800
— segmented neutrophiles	52%
— lymphocytes	45%
— moderate anisocytose	

Dissection of this animal revealed enlarged (congested) spleen and lungs, slight brain congestion, fatty peritoneum, full stomach, thin intestine nearly empty, and a normal colour of the kidneys.

While the stomach and lungs exhibited minor, negligible damage, the kidneys manifested different cytological alterations both in the cortex and the medullary substance. The nephron segments were particularly affected, but modifications also occurred in the connective tissue and blood vessels. The lesions were occasionally quite serious and widened spaces appeared in the kidneys. The renal corpuscle was less affected than the other segments of the nephron. However, the corpuscle revealed an enlargement of the intercapsular space and a disruption of the visceral epithelium of Bowman's capsule. The glomerulus was either destroyed with the appearance of a granular contents, or converted into a hyaline mass. The proximal tubule was occasionally more

severely affected than the other segments of the nephron. The lesions observed were:

— The epithelial arrangement was wrecked. The cells were destroyed either entirely or partly. Cell limits had disappeared. Also, the nucleus were pycnotic for the most part.

— Some cells were hypertrophied, with the obstruction of the lumen.

— In other areas the cells were flattened and the lumen was full with hemorrhagic outflow.

— At the apical of the cells, the brush border of microvilli had disappeared, and a granular mass appeared in the lumen.

Cell necrosis was also observed in the thin, distal tubules, and a little also in the collecting duct. In the distal tubule the nuclei were pycnotic, the cells appeared destroyed, and a granular mass similar to that observed in other segments occurred in the lumen. The blood vessels looked completely destroyed and wide areas were flooded with blood. The vessels were considerably enlarged in other areas of the kidney. The lesions of the corpuscle and of different other nephron segments led to a reduction in the glomerular filtration barriers and a strong disturbance of the absorptive and resorptive processes. The phenomena were followed by functional evidence of injury manifested as proteinuria.

CONCLUSIONS

The blood test of the second rodent revealed granular cytopenia and acute leukopenia, proving the radiochemical noxiousness of uranium, its chemical toxicity being revealed by toxic granules. Medullary hypoplasia or even aplasia is assumed to occur — a phenomenon that needs to be further investigated. Vitamin therapy has turned up to be beneficial leading to a nearly normal blood cell contents and an extension of lifetime. Cellular alterations were found especially in the proximal tubule, which appeared to be more severely affected than other parts. The modifications were similar in all segments, i.e. pycnosis of the nuclei, cellular necrosis, appearance of either granular mass, or hyaline in the tubule lumen or in the blood vessels. The appearance of uremia in rats subjected to internal contamination with uranium occurred by generalised necrosis, which affected the function of the nephron.

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IN VIVO STUDY OF CHONDROITIN SULPHATE UPTAKE AND DISTRIBUTION IN OOCYTES OF FROG, *Rana ridibunda*

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Chondroitin sulphate (Mr ~ 35.000) isolated from bovine tracheal cartilage labelled with fluorescein isothiocyanate (FITC) was used to study the uptake and distribution into developing oocytes of frog, *Rana ridibunda*.
Fluorescein-labelled chondroitin sulphate accumulated in pre- and vitellogenic follicles.
In young previtellogenic follicles acumulation was observed in cortical cytoplasm, probably endosomes.
In the middle and late previtellogenic follicle the CS-FITC was observed in endosomes that appear as very bright fluorescence spots at the cell periphery.
Distribution of chondroitin sulphate in cytoplasm of these oocytes was polarized.
In the vitellogenic follicles fluorescent chondroitin sulphate accumulates in cortical cytoplasm and perinuclear.
In vitellogenic follicles that internalized chondroitin sulphate, fluorescence was detected in the nucleoplasm and nucleoli.

During vitellogenesis the oocyte of oviparous animals accumulates, large amounts of vitellogenin required for the embryo.

In addition to this well-known egg component, growing oocytes accumulate a variety of other compounds, sometimes in substantial amounts, which play an integral part in the proper development of the embryonic and larvar amphibians. In some cases, however, the physiological importance of these compounds is not yet known or can only be inferred from circumstantial evidence.

Amphybian eggs are known to contain protein-bound carbohydrate moieties but their exact localization and their biochemical nature have only been given rather cursory attention. For example, nearly all amphibian species show a bi-partite yolk organelle in that the crystalline core is enveloped by a superficial layer of variable thickness. The macromolecular subunits of the crystals are composed of lipoproteins such as lipovitellins or phosphoproteins such as phosvitin and phosvetts (20, 38). The nature of the external matrix has been less closely studied: usually a layer of mainly acidic mucopolysaccharides covers the core of the fully grown yolk platelet (9, 25).

It can be hypothesized that the uptake of the large molecule vitellogenin by endocytosis may not entirely exclude smaller blood molecules such as sugars, lipids, plasma proteins or even DNA (23).

The function of glycosaminoglycans (GAGs) is an important example of polysaccharide diversity and specificity, with high impact in development and

disease (8). Hyaluronan, chondroitin sulphate and heparan sulphate have all been reported to be associated with chromatin in nuclei (10, 14, 34). In addition some glycosaminoglycans are internalized and processed by alternant intracellular pathways which generate GAGs fragments. These fragments have been shown to accumulate in the nuclei (11, 16).

The present communication describes a morphological study on endocytosis of chondroitin sulphate in developing oocytes of frog, *Rana ridibunda.*

MATERIALS AND METHODS

Animals. The experiments were carried out on *Rana ridibunda* frogs obtained from the lakes around Bucharest.

Conjugation of chondroitin sulphate with fluorescein. The chondroitin sulphate (50 mg/ml) dissolved in 0.01 M carbonate/bicarbonate buffer, pH — 9.2 containing 0.15 M NaCl was incubated with fluorescein isothiocyanate (15 mg/ml) at 4°C, overnight. To remove unreacted dye, the solution of CS-FITC was filtered by filter paper and dialysed at 4°C against sodium phosphate buffer, 0.2 M pH — 7.2, overnight. The nondiffusible material was then purified by gel filtration on a Sephadex G-50 column and eluted with phosphate buffer pH — 7.2.

The fractions were concentrated by evaporation to a final concentration of 1.5 mg/ml uronic acids.

Injection procedure. Frogs was injected in the dorsal lymph sacs with 1 ml of CS-FITC. Animals were kept in aquaria at 20°C and sacrificed at 23 hrs after a single injection.

Histological procedures: Small pieces of tissue were dissected from ovaries and fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH — 7.2) for 4 hrs at room temperature. After fixation samples were rinsed with 0.1 M cacodylate buffer, dehydrated in ethanol and embedded in paraffin. The sections were examined in a Zeiss fluorescence microscope.

Biochemical characterization of chondroitin sulphate. The following investigations for chondroitin sulphate were performed:

— uronic acids according to Bitter and Muir's method (2);
— hexosamines according to Elson-Morgan's method (7);
— total nitrogen according to Kjeldahl's method (6);
— total sulphate estimated by the gravimetric method (24);
— molecular weight according to viscosimetric method (28).

RESULTS

To determine the cellular site of uptake, FITC-chondroitin sulphate was injected in the dorsal lymph sacs and sections of the ovary were examined in the fluorescence microscope.

Chondroitin sulphate consists of alternating D-glucuronic acid and N-acethyl D-galactosamine units, of which the latter normally is O-sulphated in the 4 and/or 6 position (22).

Table 1 shows the biochemical characteristics of chondroitin sulphate isolated by us from bovine tracheal cartilage and used for endocytosis experiments.

The ovary of frogs used in the experiment was characterized by the presence of pre and vitellogenic follicles.

Table 1

Analytical determination for chondroitin-sulphate extracted from bovine tracheal cartilage

Determination	Chondroitin-sulphate
Uronic acids*	27.5
Hexosamines*	17
Total nitrogen*	3.9
Sulphate*	7.2
Average mol. wt.	35.000

* The values are related to % of weight of dry substance

In young oocytes the fluorescence appears in the cortical cytoplasm, probably endosomes (Fig. 1). In the middle and late previtellogenic follicle the chondroitin sulphate-FITC was observed in the endosomes that appear as very bright fluorescent spots (vesicles) at the cell periphery (Fig. 2). Moreover, these oocytes accumulate the dye much more rapidly (or even exclusively) in their vegetal hemisphere than in their animal one (Fig. 3).

In the vitellogenic follicle the distribution of fluorescein-labelled chondroitin sulphate appears different. Thus, in the early vitellogenic follicle, the fluorescence was observed both in peripherally located small vesicles and perinuclearly (Fig. 4). It may be noted that in middle vitellogenic oocytes the fluorescence was found also perinuclearly (Fig. 5). In some oocytes, the internalization pattern was also polarized (Fig. 6).

Uptake of fluorescent ligand was significantly higher in the middle and late vitellogenic follicle where the fluorescence was localized in the cortical cytoplasm and between yolk platelets (Fig. 7). In these oocytes the fluorescent material occurs at the surface of some platelets (Fig. 8).

Apart from these localizations a weak fluorescence (spots) was identified in the nucleoplasm and nucleoli of vitellogenic follicles (Fig. 9).

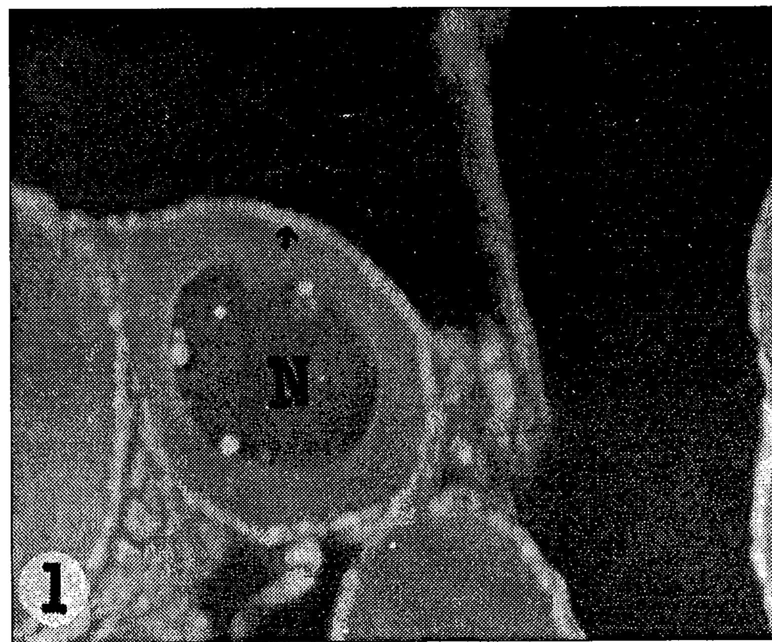


Fig. 1. — The accumulation of CS-FITC in cortical cytoplasm of early previtellogenic follicles (arrow); nucleus (N).

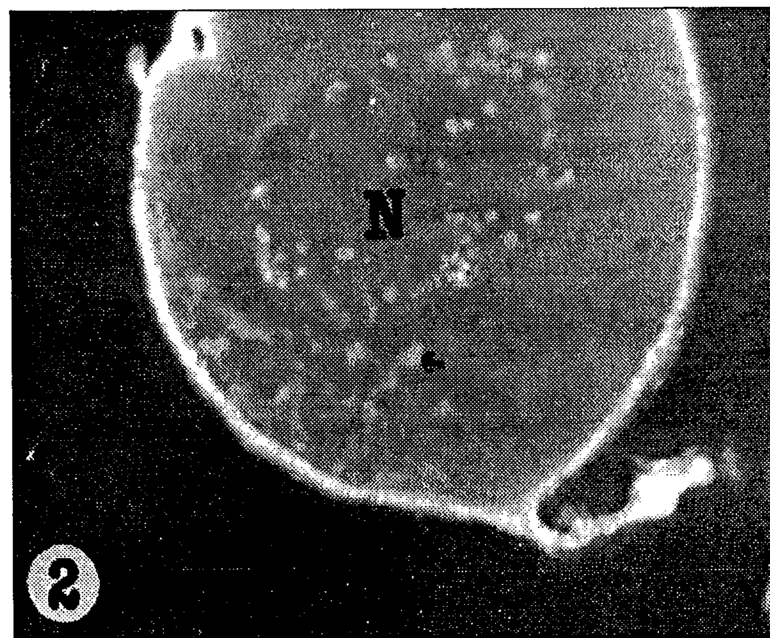
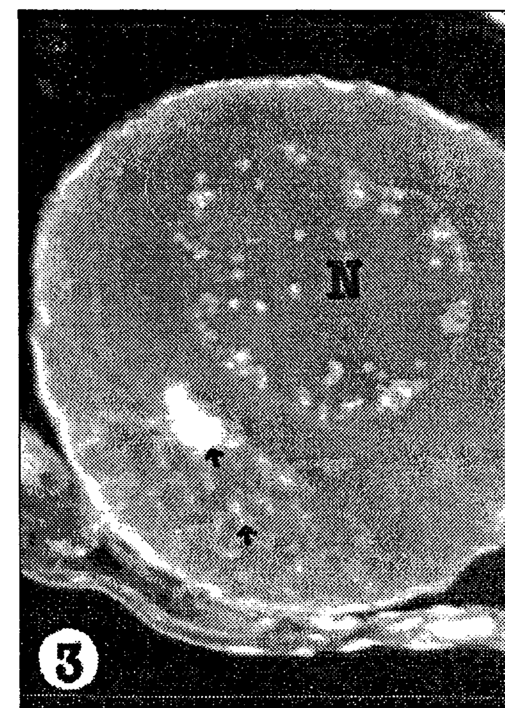


Fig. 2. — Localization of CS-FITC in vesicles at the cell periphery and in the vegetal hemisphere (arrow); nucleus (N).

Fig. 3. — The late previtellogenic follicles accumulate CS-FITC much more rapidly (or even exclusively) in their vegetal hemisphere (arrow); nucleus (N).



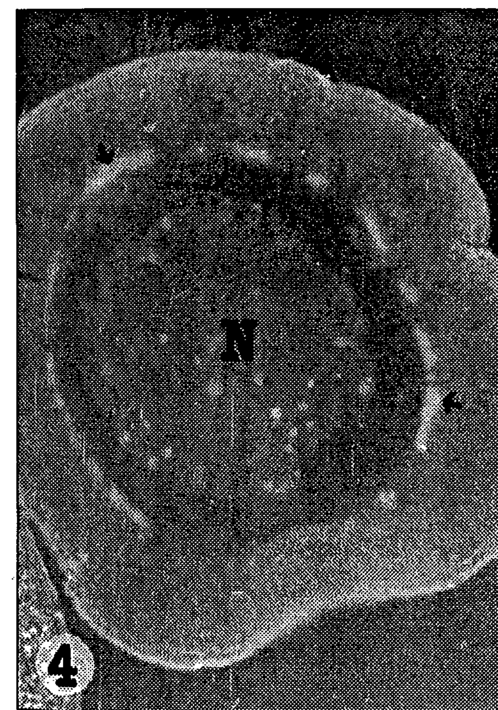


Fig. 4. — In the early vitellogenic follicle the fluorescence was observed both in peripherally located small vesicles and perinuclearly (arrow); nucleus (N).

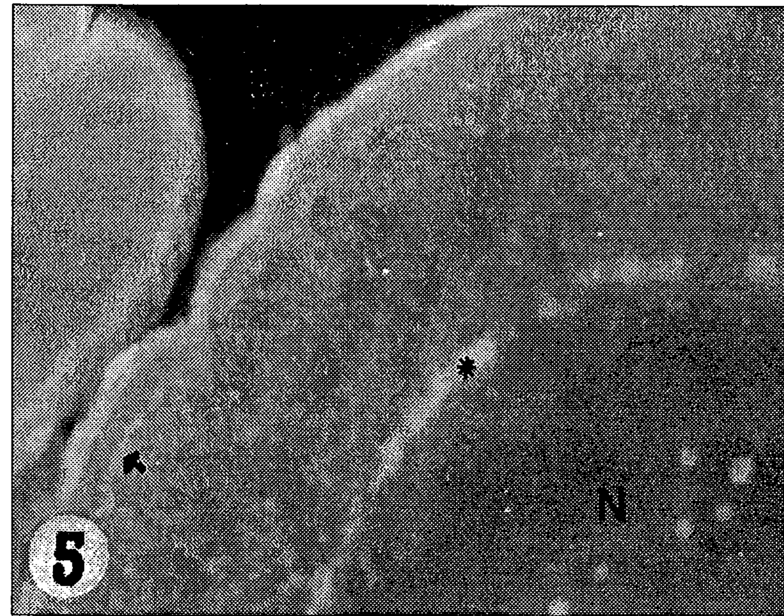


Fig. 5. — In the middle vitellogenic follicle the fluorescence was found in cortical cytoplasm (arrow) and perinuclearly (*); nucleus (N).

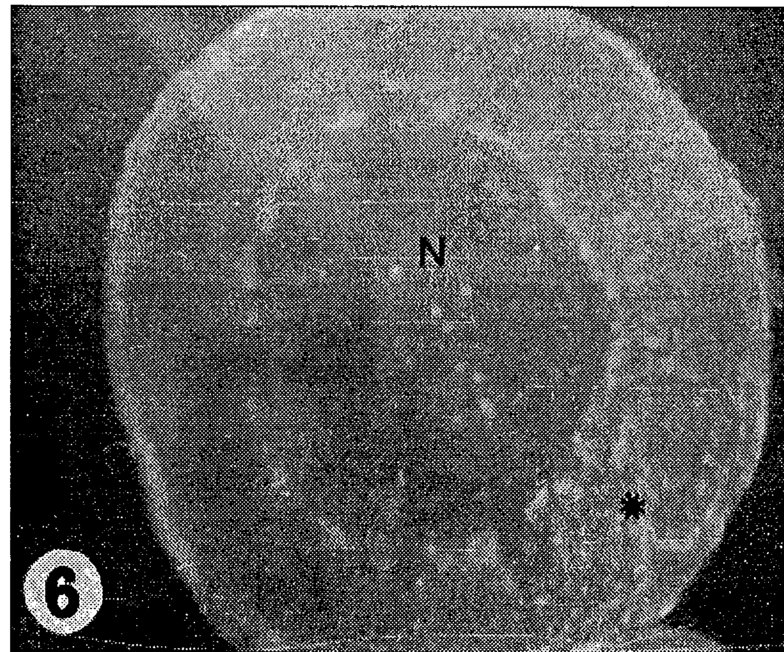


Fig. 6. — In some early vitellogenic follicle the internalization pattern was polarized (*); nucleus (N).

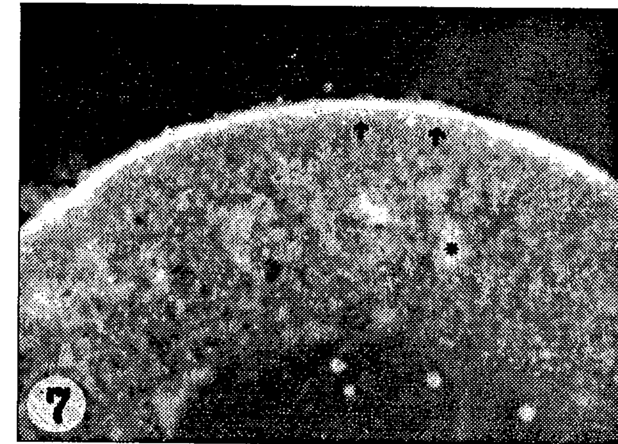


Fig. 7. — In the late vitellogenic follicle the fluorescence was localized in cortical cytoplasm (arrows) and between yolk platelets (*); nucleus (N).

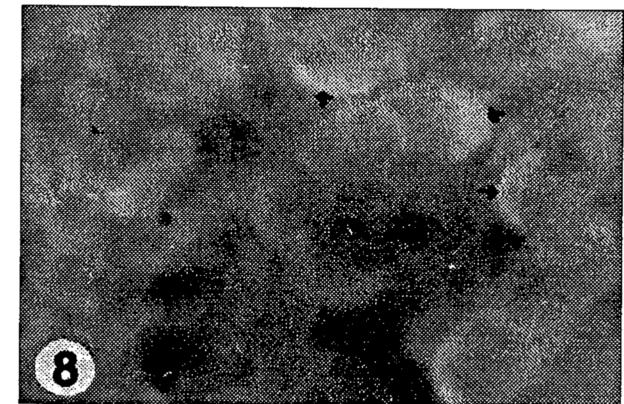


Fig. 8. — The fluorescence material was observed at the surface of some platelets (arrows).

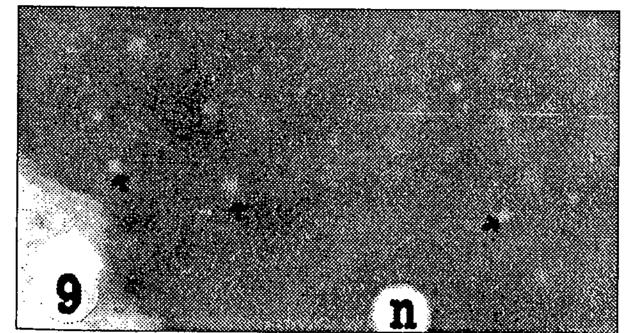


Fig. 9. — A weak fluorescence (spots) was identified in the nucleoplasm and nucleoli of vitellogenic follicles (arrows); nucleolus (n).

DISCUSSION

It has generally been assumed that the oocyte is a storage depot for many of the macromolecular components required by the developing embryo for its metabolic and nutritional needs as well as for rapid cellular proliferation.

Yolk formation in the amphibian oocyte, the origin of the yolk-protein species, storage of yolk during embryogenesis (18) and its utilization during embryogenesis (17) are not yet completely understood. The yolk platelet is composed of a crystalline core, a noncrystalline superficial layer and a bounding membrane (20). The limiting membrane of yolk platelets compartimentalizes storage substance, and the plateles become reservoirs for a variety of biomolecules such as aminoacids and protein species (37, 40), lipids and carbohydrates (27). This suggests a variety of active-transport systems in the yolk-platelet membrane (26) but further investigation is required.

In our uptake experiments the chondroitin sulphate – FITC was observed at the surface of some platelets. Athough the external matrix of the yolk platelets contains mucopolysaccharides (25) the oocyte glycoproteins and carbohydrates were associated mainly with soluble fraction of the egg, namely the cortical granule (38).

In other amphibian, *Triturus cristatus* (in which no cortical granules are ever formed), we have identified by electrophoresis a chondroitin-sulphate-like GAG in yolk extracts (Zărnescu, unpublished data).

It's known that many cells taking up GAG_S by endocytosis and internalization of these molecules are followed by intralysosomal degradation. Previous observations performed in mammalia indicated that GAG_S and proteoglycans are efficiently internalized in vivo and in vitro by a variety of cells. These studies demonstrated the uptake of hyaluronan by liver sinusoidal endothelial cells (5, 21, 31), Kupffer cells (1), decorin by bovine aortic endothelial cells (15), rat liver endothelial cells (33) and chondroitin sulphate also by liver endothelial cells (19, 21). In vivo studies have shown that GAG_S are taken up by the liver (12, 13, 19, 32), the spleen and the kidney (12, 13).

Our microscopic findings demonstrated that chondroitin sulphate – FITC was accumulated within all developing oocytes of frog *Rana ridibunda.*

The natural metabolism of GAG_S in animals is carried out by hydrolases. Generally, the GAG_S are degraded in a two-step procedure (8, 29). First, the proteoglycans are internalized in endosomes where an initial depolymerization of the GAG_S chains takes place. This step is mainly endolytic and yields oligosaccharides. Further degradation is carried out after fusion with lysosomes, a more acidic compartment of the cell where desulfation and exolytic depolymerization to monosaccaharides take place (8). Once in the lysosomes the chondroitin sulphate is subjected to the degradation sequence of hyaluronidase, β-glucuronidase and βN Acetylhexosaminidase with lysosomal sulphatase (35) acting

at the level of oligosaccharide sulphate to release inorganic sulphate. It has been suggested that the degradation of chondroitin sulphate proteoglycan is initiated by a limited proteolysis of the core protein with the production of non-aggregating species that may be eliminated from connective tissue by diffusion into the circulatory system (30, 39). In support of this hypothesis chondroitin sulphate has been demonstrated in the blood plasma of normal individuals (3).

Oocytes have lysosome-like organelles of unusal enzymatic composition at all stages of their development and the amount of hydrolase activity increases steadily throughout oogenesis. These unusual lysosomes appear to be located primarily in a peripheral zone of oocyte cytoplasm (36). In the *Xenopous laevis* the smallest oocytes had punctate foci of β-glucuronidase at the extreme periphery of the cells. As oocyte size increased the intensity then decreased.

It has been clearly documentated that vitellogenin and other serum macromolecules are delivered to lysosomes (multivesicular bodies) but that lysosomes in oocytes (at least vitellogenic oocytes) are modified so that yolk degradation does not occur (36).

Absence of degradation (or incomplete degradation) of chondroitin sulphate – FITC could explain the presence of fluorescence at the surface of yolk platelets observed in our experiments. In early previtellogenic oocyte the fluorescence was detected only at the periphery of cells while in late previtellogenic follicle and early vitellogenic ones the chondroitin sulphate – FITC is present in cortical cytoplasm and perinuclearly.

Our results indicate that in some previtellogenic and early vitellogenic oocytes the distribution of CS-FITC in the cytoplasm was polarized. Large amphibian oocytes are characterized by a typical animal-vegetal polarity which concerns most of the structural and biochemical components of the cell. The origin of this polarity is still largely unknown. Previous studies (4) indicated that incorporation of Trypan Blue is polarized in most vitellogenic stages and it varies considerably according to the size of the oocytes, The main conclusion of this study is that the vegetal half of the oocyte is elaborated early in oogenesis (4).

Apart from the cytoplasmic localizations we observed a weak fluorescence (spots) in the nucleoplasm and nucleoli of vitellogenic follicles.

In rat ovarian granulosa cell (16, 41, 42) some proteoglycans are degraded after internalization by two distinctly different pathways. In the first pathway proteoglycans are degraded rapidly without generating appreciable amounts of degradative intermediates. In the second pathway proteoglycans are degraded more slowly, associated with the generation of distinct degradative intermediates.

In conclusion our microscopic findings indicate that chondroitin sulphate – FITC is internalized by all developing oocytes of frog *Rana ridibunda.* The functional role of this GAG in frog oogenesis should be further investigated.

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SEASONAL OXYGEN CONSUMPTION IN THE GOLDFISH *(CARASSIUS AURATUS GIBELIO* BLOCH)

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Oxygen consumption in goldfish *(Carassius auratus gibelio* Bloch) presented a seasonal variation in the period September (I) – August (II) in direct dependence upon temperature variation in conditions of natural environment (Dridu ponds). Thus the lowest values were recorded in winter months (e. g. in February 55.95 ml O_2/kg/hour at the temperature of +0.34°C), while the highest in the summer months (e. g. in August 300.61 ml O_2/kg/hour at the temperature of +27.5°C).
By the statistical adjustment, to the conditions of constant temperature (+24.78°C +/–0.20°C) the oxygen consumption curve reached a low level during the winter (except for December), a high value at the beginning of spring, after which it decreased gradually till June, when the second depression was registered (less accentuated than the winter one). At the end of summer oxygen consumption gradually increased with slightly higher values being recorded during the autumn months and continuing until December.
Seasonal variations of metabolism, independently of the reproductive factor, were studied by using specimens of fishes immature from the sexual point of view.
The author discusses the obtained results in connection with present knowledge about metabolic rhythmicity in fish.

INTRODUCTION

Seasonal rhythmicity of energy metabolism in aquatic poikilotherms was much less studied than nyctimeral rhythmicity (circadian, 24-hour rhythm, etc.) of the metabolic function in this group of animals. It is generally considered that the seasonal factor comprises a diversity of aspects, among which the essential role would be played, primarily, by temperature. As the influence of the temperature on energy metabolism constitued, already in the last century, the object of numerous investigations, it is essential that this influence be removed in view of evaluating a seasonal rhythm independently of the temperature factor.

The studies undertaken in the sense are scarce, and Wells' researches (30) on the Pacific fish *Fundulus parvipinnis* are considered as the first indication of this kind in literature, though before him Knauthe (quoted from 1) observed in the carp a variation of oxygen consumption in conditions of a near temperature. 24, 13 and 26 admit the existence of a seasonal metabolism rhythm independent of temperature without the support of this acceptance any other work but that of Wells'. 6, 18, 22, 16, 31 and 32 note seasonal variations of oxygen consumption of fishes.

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In our experiments we proposed the evidencing of a seasonal rhythm of oxygen consumption in the species *Carassius auratus gibelio* Bloch., by the parallel examination of the influence of seasonal modifications in both field conditions and laboratory conditions, where the temperature was kept constant throughout the year.

MATERIAL AND METHODS

Experiments were carried out on the goldfish *(Carassius auratus gibelio* Bloch). The necessary specimens were collected from the ponds Dridu. Batches of fishes of 15–25 specimens were used. Body weight was between 10–45 g. Analyses were achieved by measuring the oxygen consumed in a closed chamber, from which more than 25–35% of the available oxygen quantity was never consumed.

Oxygen analyses were made according to the Winkler chemical method, and experimentations were effected in the interval between 9–16 hours, so as to avoid as much as possible the influence of nyctemeral variations.

Field experiments were executed monthly, where aquariums were placed on the shore of the pond and filled with pond water in order to account for the aggregate of ecoclimatic and chemical factors of the natural sea. Fishes caught with the net were used in field experiments after an interval of several hours (2–4), during which they were kept in an accommodation aquarium to avoid behavioral reactions caused by the manipulation of fishes. This time interval is long enough for the disappearance of behavioral influences, as was shown by Motelică's experiments (19) on emotional hyperglycemia in the carp.

In the laboratory, batches of fishes collected in the same month from the Dridu ponds were used, after a previous (3–4 days) adaptation (acclimation, 14) at a constant temperature of 24.78°C +/–0.20. Constant temperature was achieved throughout the experimentation year, by the use of a relay installation adapted to the test aquarium.

In view of eliminating the variability among sexually mature fish, we used sexually immature specimens in our experiments.

RESULTS

Experiments were effected each month, concomitantly in the field at ambient temperatures and in laboratory conditions, at a constant temperature.

I. *In the field.* Oxygen consumption values of the goldfish, during the months of September (I) – August (II) are represented in Table 1, in which average temperature, average weight of the batch of fishes, average oxygen consumption for the respective month, as well as the number of determinations are indicated.

Table 1

Seasonal oxygen consumption in field conditions

Date	No. of fish	Average temperature °C	Average weight	Average O_2 consumption in ml. O_2/kl/h
7 Sept.	17	20,71	22,97	225,56
21 Oct.	20	16,26	21,71	196,95
15 Nov.	21	6,10	31,03	92,26
20 Dec.	16	0,54	35,32	61,44
10 feb.	10	0,34	34,74	55,95
14 Mar.	12	6,50	27,67	93,95
18 Apr.	19	10,76	25,79	111,62
21 May	22	22,31	26,68	221,08
15 June	23	19,33	28,88	202,07
8 July	17	24,09	26,50	247,51
21 Aug.	11	27,50	28,55	300,61

For evaluating the relationship between oxygen consumption and temperature in the natural conditions of the pond complex in the studied species, a graph was drawn up in which curves representing the monthly values of the two parameters are presented (Fig. 1).

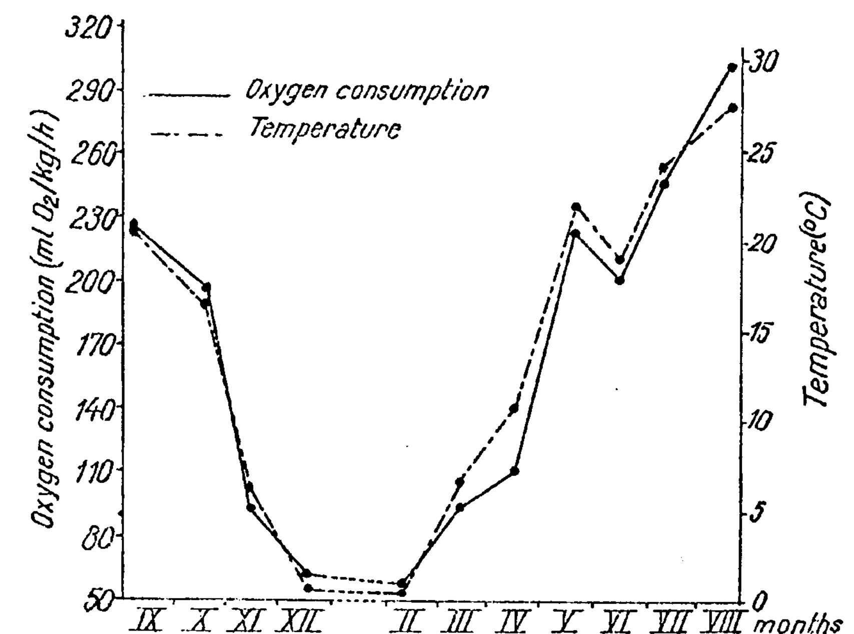

Fig. 1. — Seasonal oxygen consumption in goldfish *(Carassius auratus gibelio* Bloch) in the field conditions.

II. *In the laboratory*. Experimental results under constant temperature are presented in Table 2.

Table 2

Seasonal oxygen consumption at annual constant temperature

Date	No. of fish	Average temperature °C	Average weight	Average O_2 consumption in ml. O_2/kg/h
12 Sept.	15	25.29	23.51	251.53
27 Oct.	20	24.78	24.12	254.36
23 Nov.	22	24.91	20.28	255.12
24 Dec.	15	24.64	26.39	252.54
15 Feb.	11	24.94	40.14	170.39
22 Mar.	22	24.91	17.73	287.10
25 Apr.	25	24.50	24.14	265.51
27 May	19	24.57	20.68	238.38
22 June	14	24.60	18.49	214.56
12 July	17	24.81	22.54	245.58
28 aug.	15	24.80	20.32	257.45

On the basis of averages for each month, a graph was drawn up showing a seasonal variation of energy metabolism (oxygen consumption) of the species studied during the months of September (I) – August (II) in constant temperature conditions (Fig. 2).

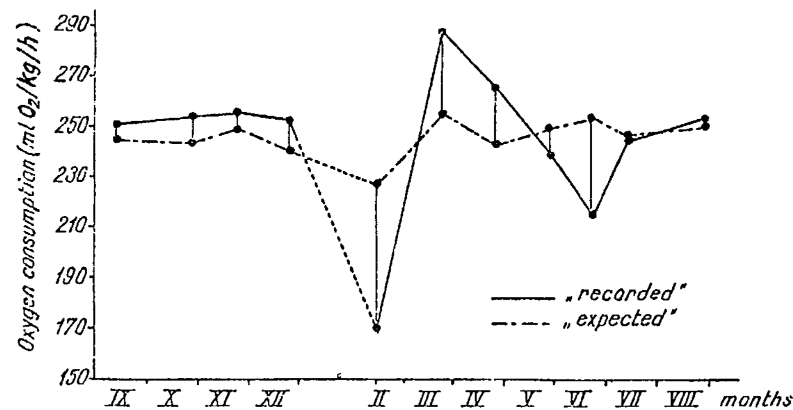

Fig. 2. — Seasonal oxygen consumption in goldfish *(Carassius auratus gibelio* Bloch) in constant temperature conditions.

Statistical correction of data was effected in order to eliminate body weight difference between the different batches of fish. For this purpose all experimental data were statistically worked out the regression straight line coefficient (b = 0.860) — the weight exponent — was determined.

By using the classic equation of the relationship between oxygen consumption and body weight ($Q = K{\cdot}Pb$), in which Q represents metabolic magnitude (the produce between oxygen consumption and weight in 24 hours); K = constant; P = body weight and b = weight exponent (regression coefficient), determined by computation to have the value of 0.860 the corrected ("expected") values, corresponding to the weight of the batch utilised in the respective month were obtained (17). The comparison of the new values obtained by computation with those resulted directly from experiment, permitted the establishment of the percentage variation of real values as against the corrected ones (Table 3).

Table 3

Seasonal variation of the real (corrected) oxygen consumption in goldfish at constant temperature

Date	Recorded value	Expected value	Percentage difference
Sept.	251.53	244.93	+ 2.69
Oct.	254.36	244.01	+ 4.25
Nov.	255.12	249.95	+ 2.07
Dec.	252.54	240.98	+ 4.80
Feb.	170.39	227.20	– 25.01
Mar.	287.10	254.85	+ 12.65
Apr.	265.51	243.96	+ 8.83
May	238.38	249.31	– 4.38
June	214.56	253.31	– 15.30
July	245.58	246.35	– 0.32
Aug.	257.45	249.93	+ 1.03

The corrected values are in Fig. 3. An accentuated depression of oxygen consumption level during winter (February), followed by an intense increase in

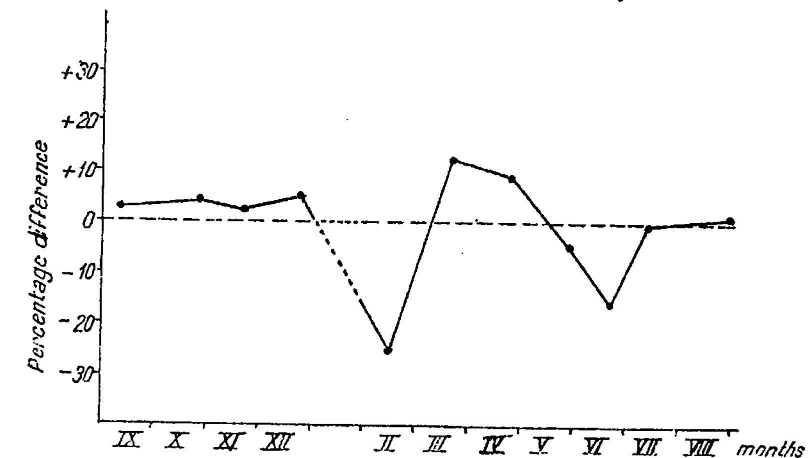

Fig. 3. — Seasonal variation of the real oxygen consumption in goldfish *(Carassius auratus gibelio* Bloch) in constant temperature conditions.

the spring period (end of March – April) was recorded. At the end of May and, especially, in June, a decrease is recorded, after which the curve approaches the average value (the second half of July and particularly the end of August). The autumn period marks a slight increase of the oxygen consumption level (4.80% at the beginning of December).

DISCUSSION

The oxygen consumption curve of *Carassius auratus gibelio* Bloch in conditions of constant temperature has two depressions: one in the winter period (first part of February — in January no determinations were made) and a second one in June. In the case of the first depression, the decrease recorded is reflected in the relationship between the real value and the computed (expected) value, and represents –25.01%; depression of May and June reaches a maximum value (–15.30%) in June.

In other months of the year, the accentuated increase of the level of metabolism at the end of March and the beginning of April (+12.65% and +8.83% respectively) is worth mentioning. In autumn months and in the first part of winter (December) a minimum but constant increase of oxygen consumption is recorded, which points out an increase of metabolic requirements in this period, though much lower than that in spring.

The results obtained by us are comparable with those shown by Wells (30), who recorded a significant increase in spring, followed by a decrease of metabolic intensity in the warm season. The high values pointed out by Knauthe's results from 1 in the months of April–June, accompanied by diminution of oxygen consumption in the carp in the months of September – October, and, especially those obtained by Beamish (6), show a minimum in the spring months (April – May) which reaches a value half the maximum level during the autumn months, do not represent the occurrence of insoluble contradictions as regards the explanation of seasonal variations of fish metabolism. Knauthe investigated seasonal fluctuations (we can admit temperature limits of 16.7°–17.6°C as sufficiently close) in the case of the carp, while Beamish investigated two trout species. The maximum values recorded by both authors are expressed exactly in the period in which the respective species were in the breeding period: carp in the spring — early summer months, while trouts in the period of November to the middle of January. Moreover, Beamish notes the variation of the occurrence of maximum values in relation to the breeding period, which occurred differently in two consecutive years: November respectively December.

The correlation of metabolic maximum with the breeding period are recorded also in other aquatic poikilotherms. Thus in the fresh-water snail *Ancylus fluviatilis*, were found (7) the highest values in March and May, when these

animals were entering the breeding period, while other authors (20) note the diminution of the respiratory rate in the *Patella vulgata* snail, simultaneously with the onset of the "post-reproductive" period.

In our determinations we had in view to avoid the influence of this factor, which seems to be determinant in the increase of metabolic value. From this point of view, our results are not inconsistent with the above indicated ones, as by their size and body weight (< 45 g), the specimens used were always before sexual maturity. The goldfish is a species in which reproductive maturity is established at about 2 years, more seldom at 13–15 months, which would correspond to a weight of about 60–80 g in conditions of a water in which the youth of this species is formed by a ginogenesis strongly influenced by the presence of the carp (9, 4).

Wohlschlag and Juliano (33), in an ample field study of the metabolism of the fish *Lepomis macrochirus*, in which the multiple regression coefficients for weight, activity and temperature are analysed, consider the increase of oxygen consumption in the spring season — similar to the results obtained by us for the same period — as being conditioned in nature by the increase of temperature, activity and, probably, of the secretion of stimulating gonadal hormones. Wohlschlag et al. (32) have investigated the respiratory metabolism of the pinfish (*Lagodon rhomboides*) under the influence of season. The summer and winter levels for "routine metabolism" indicated definitely that winter values were higher at all temperatures than the summer levels. These values are in a good agreement with our results for the constant temperature. According to Hoar (15), the endocrine modifications associated with the development of gonads and spawning would influence metabolism considerably. Data concerning the relationship between thyroid activity and oxygen consumption in fishes are mentioned in literature. These data have, however, a contradictory character (6, 3, 25).

Huxley, (27), considered that thyroid gland may act as a mechanism for temperature influence compensation. As regards thyroid activity in relation to season, the seasonal variation of this gland is correlated by most authors, with the sexual maturity period, a marked increase of specific activity, as well as of some structural indices being recorded during reproduction. In addition, Swift (27, 28), utilizing sexual immature specimens of *Salmo trutta*, notes a rise of thyroid activity as well an increase of the height of these cells, followed by its diminution until July, with a subsequent rise of a small value, which were maintained until the first part of winter. This evolution would correspond to the oxygen consumption curve obtained by us in conditions of constant temperature, except for the values during winter (1-st half of February). We deem that this negative correlation between the thyroid activity curve, mentioned by Swift and

that of oxygen consumption obtained by us is determined by the strong influence, in wintermonths, of low temperature upon metabolism.

Eales (12) considered that other variables, besides temperature and photoperiod, can affect thyroid activity within the season. In experiments undertaken on the activity of this gland in *Salmo gairdneri* in one year old specimens, he notes a growth of cell height during spring, which decreases at the end of summer, attaining a moderate value during winter. In contradiction with the height of thyroid cells, radioiodide indices increase in spring, reach a maximum in late summer, and decline in winter. This latter thyroid activity curve (on the basis of the radioiodine criterion) coincides with the variation of oxygen consumption found by us in *Carassius auratus gibelio* Bloch. In this sense correlated studies concerning oxygen consumption (as a metabolic index) and the activity of some endocrine glands, whose influence might intervene in metabolic processes, in relation to season, are necessary.

Recently (31) the interactive effects of season and temperature on metabolic functions in *Rutilus rutilus* L. have been examined. The authors found big differences between oxygen consumption values in individuals acclimated to a constant temperature for 1 year and the others acclimated for only 14 days.

The idea was suggested that beside other factors which can be comprised in the notion of the wider sense of season, an important role in the release of metabolic modifications during an annual cycle might be attributed to food in the natural habitat. Thus, the increase of metabolism in the spring season coincides with the raising of the thermic level, of activity, of hormonal secretion, beside feeding intensification in conditions of an increase of the quantity of food. Barnes et al. (5), assessing oxygen consumption in two species of *Balanus,* found that respiration intensity, which is maintained at a low level during winter, rises in early spring, simultaneously with the increase of body weight, of reserve materials and of the total content in ribonucleic acid. Barry and Munday (quoted from Davies, 11) ascertained in the limpet *Patella* an increase in the concentration of blood sugar and tissue reserve glycogen, similar to the respiration increase in the spring season.

Marinescu and Künnemann (16) have recently found that individuals of *Idus idus* L. acclimated at 25°C had different values of oxygen consumption in two spring months (March and April).

We likewise mention the seasonal variation of total nitrogen excretion, obtained by Picos and Marinescu (21) in the carp, in conditions of inanition and within close temperature limits (18–20°C). They established an increase of the nitrogen quantity eliminated during the spring, followed by a progressive decrease during the summer and autumn season, the lowest value being recorded during the winter. Except for autumn values (when the diminution of the excreted quantity of nitrogen can be accounted for by the increase of metabolic (anabolic)

processes of reserve materials incorporation) the nitrogen excretion curve is comparable to the one found by us for oxygen consumption of the goldfish in conditions of constant temperature.

The diminution of metabolism during the summer may be due both to the adaptation to "stress" conditions, determined by the high temperatures of this period of the year, as well as to a diminution of the quantity of food. In the conditions of these ponds in the warm season (June and first part of August), a quantitative decrease of the phyto — and zooplankton is recorded (10), which may account — as a local adaptation — for the diminution of oxygen consumption in goldfish, recorded by us in this period of the year.

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RESEARCHES REGARDING USE OF *OSTRINIA NUBILALIS* SYNTHETIC SEXUAL PHEROMONE IN ROMANIA

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Investigations presented were undertaken to develop the foundation for the future control of the European corn borer, *Ostrinia nubilalis* Hb. with a pest management system based on pheromone. The paper presents the results obtained in Romania during 1990–1995 in field trials with pheromone formulations development for European Corn Borer *(Ostrinia nubilalis* Hb.). There is a relatively good enough formulation of Z and E sexual synthetic pheromone, this formulation being not so efficient and specific. It is stated that in Romania Z pherotype is predominant all over the country. Pheromone traps can be used to draw up flight curves of *O. nubilalis* males. Control of ECB by mass trapping of males or male disorientation has failed in a small corn field surrounded by a forest.

Pheromone trap is helpful in a future integrated control system of pest by determination moment of releasing *Trichogramma* spp., or males with inherited sterility.

Results had determined the conclusion that using of pheromone traps has to be a component of an integrated control system.

A simple and efficient means for monitoring ECB populations is requisite for integrated pest management (IPM) programs and pheromone traps seem to be one of them.

The ECB pheromone, isolated by Klun (1968), consists of (Z-) and (E-) 11 tetradecenyl acetate (2).

From that time a lot of researches have been done referring to the pheromone structure population in different parts of the world (3, 4, 7, 14).

The European corn borer is considered in Romania the most important pest of maize crop after panicle appearance, being spread throughout the cropping zones in the country. Damages caused by this pest as grain can reach sometimes up to 40% (5). Multiannual data indicated averages of 44% plants attacked, 1.1 larvae/plant, 23.180 larvae/hectare and 550 kg/ha yield loss or 7.5% (6). The pest develops one generation per year, except for zones in the south, where partial second generation occurs. The population of this second generation is less than 20% of the first generation and it is without any economical importance. Its importance results only in maintaining a high level of pest populations. The mass flight of moths of the overwintering generations commonly takes place a few days before panicle emergence, from middle of June to first 10 days of July for the southern part of the country and from 10th July to the end of the month, for the central and northern part of the country. The main

attack sites are the larval tunnels inside the stems. The attack on maize is economically important. However, the pest also feeds on hemp and sorghum crops as well as on various species of wild flora. Due to its outstanding economic significance for maize crop in Romania, during recent years, particular attention was paid to the study of synthetic sex pheromone (8, 9, 10) and since 1988 to the investigations on male sterilization by radiation (1, 11, 12, 13).

In Romania there is a network for forecast and warning for diseases and pest, an ECB is one of the pest which it is supervised. Actual system of forecast and warning consist in watching for minimum 3 field of different hybrids in each district of center of forecast and warning responsibility, frequency of attacked plant for 1000 plants/field and no. of larvae/attacked plant by cutting of 100 corn stems/field and it is calculated pest population/ha. It is considered that a mortality of 90% is normal during the winter period and in fields in which there were more than 20,000 larvae in the next year because the economic threshold is 2000 larvae/ha. Warning system is based on registration of flight curves made by light trap.

MATERIALS AND METHODS

During the years 1990–1995 in the scientific network of the Research Institute for Cereals and Industrial Crops-Fundulea, a series of studies refrering to the specificity and efficacy of synthetic sexual pheromone lures and traps produced by the Chemistry Institute from Cluj-Napoca, Romania has been done. Pheromone sticky traps type (F-1), suspended 1 m above ground, were used in corn fields separate from 50 to 50 m. Lures were changed at 2 weeks interval and adhesive parts anytime when it was necessary. There were registered weekly no. of target species and no. of different other micro and macrolepidopterous trapped. Field tests were conducted from June to September.

In order to develop an insect pest management, special attention was paid to the investigations on the ECB dynamics and the estimation of the natural population by means of pheromone traps and markers. It is obvious that information on the flight of ECB is highly important and that knowledge of the dispersion and flight range of any insect in all its aspects is meagre indeed and for this reason a great part of our researches were directed towards establishing the distance to which a moth may fly. Dye marking method is usually made with an emulsification mixture of Calco oil red N-1700 (150 mg/kg diet) and sunflower oil, mixed homogeneously in the agar of the semisynthetic diet for ECB (13). The larvae and moths were marked after eating the diet.

Releasing and recapture in pheromone sticky traps of marked moths were used to study the behaviour of ECB males, released in the field and recaptured in pheromone traps situated at different distances far from the releasing site in

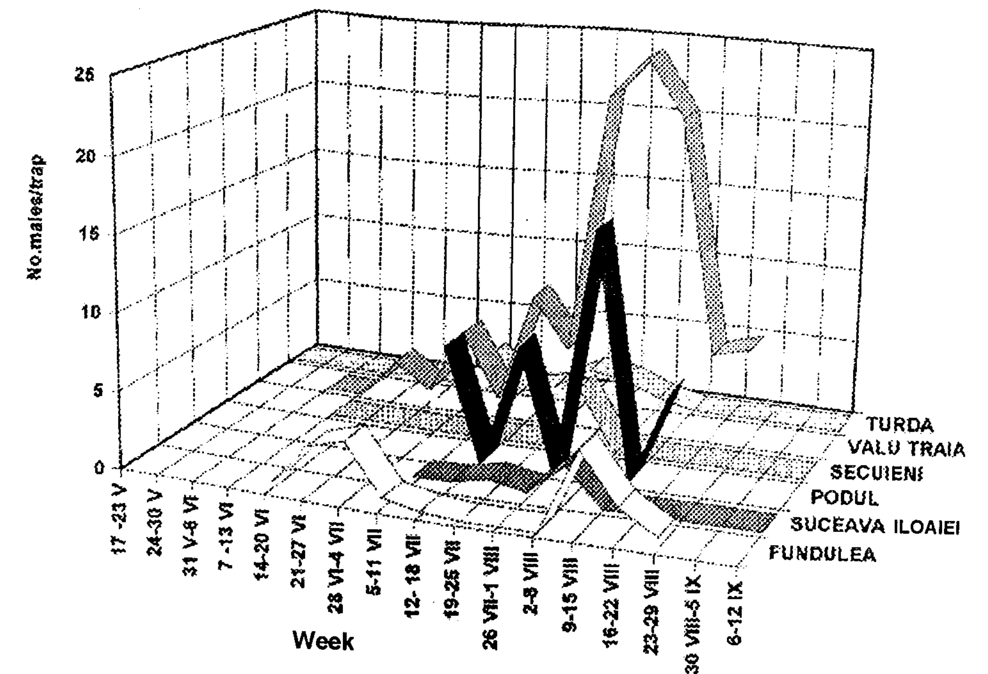

Fig. 1a. — Males flight of *Ostrinia nubilalis* Hb. in 1995 [var. Z].

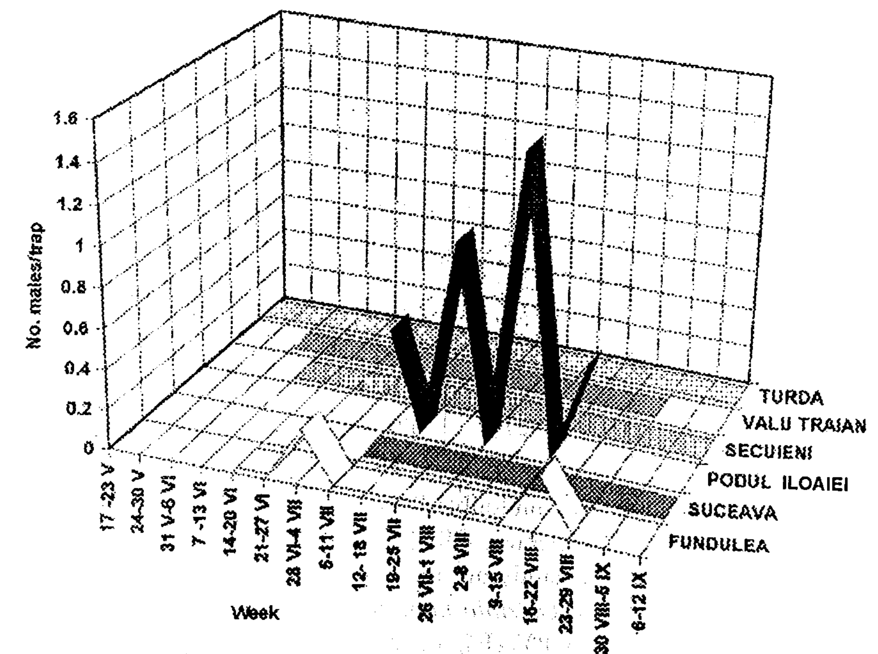

Fig. 1b. — Males flight of *O. nubilalis* Hb. in 1995 [var. E].

N, S, E and W direction. The released marked moths have to be checked individually for the red color of the abdomen.

Parts of integrated pest management were studied like: mass capture of ECB males made by 16 sticky traps/ha (25/25 m), mating disruption (male disorientation) was made by 100 *cis* (Z) lure/ha which were changed weekly during the first flight of moth (15 June – 30 July), 5 releasing of *Trichogramma* spp. was made at rate of 200,000 ex/ha at Fundulea and 3 releasing (50,000 + 100,000 + 100,000) at Turda and releasing of males with F-1 inherited sterility and normal males in the control field.

RESULTS AND DISCUSSIONS

As a consequence of preliminary results recorded during 1982–1987, 4 promising pheromone variants were found and from these we chose two, namely: E_5(*Z* 11–14OAc + *E* 11–14OAc at a ratio of 97/3) and I (Z 11-Oac + E 11–14 Oac at a ratio of 3/97) + tetradecenyl acetate (10).

The results in table 1 reveal the relatively high number of *O. nubilalis* males captured throughout the period under study, this number varying according to locality, year and pheromone variant used. The biggest number of males captured/trap (172) was registered at Turda in 1992.

Table 1

Number of males captured/year/Z-trap

LOCALITY	1990	1991	1992	1993	1994	1995
SUCEAVA	1.75	1	ND	19.5	45.5	5
PODUL ILOAIEI	ND	19	13.75	19	21.33	37.5
SECUIENI	0.5	ND	1.5	0.25	12	4
VALU TRAIAN	0	ND	13	17	32.33	104
FUNDULEA	8.25	14.75	23.25	29.5	35.25	17.2
ORADEA	5	13.5	9.75	54.33	12.1	4
TURDA	45	53	172	49.5	64.9	7.4

It is to stress that it was undoubtedly for the first time that in Romania both the pherotypes of this species, CIS (Z) and TRANS (E) exist, this later being at the beginning of this study not recorded in the center and north-east of the country. (10). A report of *Ostrinia nubilalis* pherotype showed that 92% of populations were (Z) and only 8% (E), this report being different from year and locality (Table 2).

Table 2

Report of ECB pherotype population in Romania (No. males/trap)

Pherotype	1990	1991	1992	1993	1994	1995
Var. E	6.25	9.5	34.74	11.2	19.1	7
Var. Z	60.5	101.25	233.25	189.08	223.41	179.1

Specificity of pheromone formulations are not optimal, since other microlepidoptera like *Tortrix viridana* L. in forested areas are also frequently caught and *Etiella zinkenella* Tr. and *Emelia trabealis* Scop. in the other zones; however these species can be easily separated from the target species.

Pheromone traps can be used to draw up flight curves of *O. nubilalis* males in the first generation and also in the second one in the years and localities where this appears and this fact is shown in figure 1 A, B for the 1995 year. Our results underline that the second generation generally occurs in Romania at the end of August or early in September and is practically devoid of significance, since it is numerically low, on the one hand, and during this period maize crop being to maturate, on the other hand.

Releasing and recapture of marked moths were used in order to compare behaviour of F-1 males with normal males, released in field and recaptured in pheromone traps situated at 25, 100 and 200 m in 1993, at 100, 200 and 300 m in 1994, at 300, 600, 900, 1200 and 1500 m in 1995 only for normal males and 1000, 2000, 3000, 4000 and 5000 m in 1996 only for normal males far from releasing site in N, S, E and W direction. The released marked moths have to be checked individually for the red color of abdomen. Due to the fact that recapture

Table 3

Specificity of (Z) pheromone (No. of individuals/trap)

	1990	1991	1992	1993	1994	1995
FUNDULEA						
Target species	8.25	14.75	23.25	29.5	35.25	17.2
Macrolepidoptera	2.5	3.25	4.25	1.5	2.75	2
Microlepidoptera	10.25	5.75	9.5	16.75	13.25	4.5
TURDA						
Target species	45	53	172	49.5	64.9	7.4
Macrolepidoptera	65	6	7	12	10.75	5
Microlepidoptera	12	12	25	46.11	19.5	7.25

in the pheromone traps of marked moths was almost at the same level [5.88% in 1993, 3.88% in 1994, 0.23% in 1995 and 0.25% in 1996 at control and 4.45% in 1993 and 3.66% in 1994 at males with inherited sterility in F-1] and pattern of distribution of captures has shown that dispersal capacity of moths was the same, flying of moths being no more than 300 m/day during that time of experimental conditions, it seems that there is no difference between F-1 and control moths. The response of both variants was the same regarding the synthetic sexual pheromone [*cis Z* (E-5)] and for this reason during 1995 and 1996 there were made only with control marked males.

Experiments from 1995 and 1996 have shown that released tagged ECB moths have been recaptured in pheromone traps at a distance no more than 3000 m from the point of release, and probably this is the distance at which the moths are capable to fly.

Experiments of different possible parts of an integrated control system of ECB, based on the use of pheromone traps, showed that even the lure *cis* (Z) was changed weekly and the total number of males captured was 231 in 1993, 325 in 1994 and 147 in 1995. Neither this method worked, nor disorientation of males. Reduction of population differs from year to year, sometimes being noticed an increase of population (Table 4).

Table 4

Effect of using pheromone in small isolated fields [FUNDULEA]

	Attacked stems (%)			No. larvae/attacked plant			Level of population (%)		
Variant	1993	1994	1995	1993	1994	1995	1993	1994	1995
Mass trapping of males (A sticky trap at 25/25 m)	28.3	28.0	12.6	1.12	3.1	0.75	+ 7.8	– 8.5	+ 45.8
Check	21.0	31.5	10.8	1.14	3.01	0.6	—	—	—
Male disorientation (A Z pheromone lure at 10/10 m)	22.5	46.5	18.3	0.8	2.9	1.3	– 43.7	– 5.6	+ 12.7
Check	26.0	42.0	21.1	1.23	3.4	1.0	—	—	—

Because the population of larvae has decreased after treatment with *Trichogramma* spp. and release of males with F-1 inherited sterility, and using of these methods involved pheromone traps for choosing the proper time for releasing of *Trichogramma* spp. and males with F-1 sterility, it seems that using synthetic sexual pheromones has to be a component of an integrated control system, in connection only with the release of *Trichogramma* spp., or F-1 sterility, because the use of pheromone in mass trapping or in males disorientation has no effects in experimental conditions.

CONCLUSIONS

There is a relatively good enough formulation of Z and E sexual synthetic pheromone, this formulation being not so efficient and specific.

It is stated that in Romania, Z pherotype is predominant all over the country.

Pheromone traps can be used to draw up flight curves of *O. nubilalis* males.

The control of ECB by mass trapping of males or male disorientation has failed in a small corn field surrounded by forest.

Pheromone trap is helpful in a future integrated control system of pest by determination of releasing *Trichogramma* spp., or males with inherited sterility.

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THE PESTICIDES POLLUTION IMPACT ON THE STRUCTURE AND DYNAMICS OF THE ARTHROPODS ASSOCIATION

IRINA TEODORESCU, A. VĂDINEANU

INTRODUCTION

Human activities are responsible for major, but unfortunately negative changes upon environment.

The nature conservation and sustainable development strategy lay special emphasis on the conservation of living natural resources — the biological diversity. The objectives focused on nature conservation refer to the maintenance of the essential ecological processes and genetic diversity, sustainable use of ecological systems'hierarchy.

The direct and indirect toxic actions of pesticides and industrial emissions have negative impact not only on human, but also on all trophic categories of organisms. The effects on individual and populational levels are emphasized implicitly at the biocenosis and biosphere levels, with changes in the structure and functions of ecological systems.

A special attention has to be paid mainly to heavy toxic substances, to substances with great environment remanence, and to those which are accumulated and concentrated in different trophic categories of organisms.

Since negative effects of the different pollutant upon all organisms and the tolerance limits of the species and ecosystems are not known, sustained researches are required to establish impact assessment and efficient environment monitoring.

MATERIAL AND METHODS

The investigations for the impact assessment of pesticides upon the biocenosis exposed to these aggressive factors action, were undertaken in 11–21 July 1996 in the polluted and unpolluted (control) potatoe crops, in Bucharest area.

The used insecticide was Fosalon (0.7 kg/hectare).

The samples were analysed comparatively from the polluted and unpolluted crop.

For the pesticide effect assessment, the untreated site was in the same crop, before the pesticide application.

The structure and dynamics of the ground level arthropods fauna of the potatoe crop was influenced both by the precursory crops pests (the hibernant species) and the adjacent crops (immigrant species).

A special attention was given to the influence of variation of some abiotic factors (temperature and humidity values, wind direction and intensity).

Pit-traps at the ground level, with acetic acid as attractant and preservative substance, were used for sampling. The usage of the alcohol or phormol was avoided, because the first evaporates and the latter has a repellent action.

The samples were analysed both quantitatively and qualitatively.

The qualitative analysis implies:

• identification of the material (to the species level, in most cases);

• specification of the trophic categories;

• pointing out the specific pests (for potatoe, precursory and adjacent crops) and the polyphagous pests;

• emphasizing the useful species: predators, parasitoids, necrophagous, detritophagous.

The quantitative analysis, on the other hand, includes:

• evaluation of the weight of different arthropods groups in samples;

• comparison between the numbers of species and individuals in the unpolluted and polluted crop samples;

• estimation of species numerical abundance in the unpolluted and polluted crop samples;

• following the different species frequency in samples;

• emphasing the dominant and constant species, based on the abundance and frequency values;

• comparison between the different trophic categories weight in the unpolluted and polluted samples.

RESULTS AND DISCUSSIONS

The **biocenosis structure** includes different trophic categories of organisms, such as: **primary producers** (photosynthetic and chemosynthetic organisms), **consumers of different degrees (primary,** phytophagous organisms feeding upon primary producers, **secondary,** parasitoids and predators, feeding upon the primary producers, **tertiary,** parasitoids and predators of the secondary consumers, **quaternaries,** parasitoids and predators of the tertiary consumers, **coprophagous,** which consume the catabolites of other animals, **necrophagous** fed by dead animals, **detritophagous,** that live on organic particles, resulted from the fragmentation and partial decomposition of the dead plants and animals, **omnivorous,** which consume both animal and vegetal food, **carnivorous plants,** which besides photosynthesis, complete their nitrogen requirements upon small organisms) and **decomposers,** bacteria and fungi species, which decompose dead organisms, with liberation of mineral components.

Our work did not take into consideration the primary producers and decomposers.

A research programme focused on all biocenosis organisms *(Plantae, Bacteriophyta, Mycophyta, Protozoa, Vermes, Mollusca, Annelida, Crustacea, Arachnida, Diplopoda, Chilopoda, Insecta, Amphibia, Reptilia, Aves* and *Mammalia),* on investigation of the changes of photosynthetic rates, of animal species diversity, of decomposers activities, would imply not only many researches, but also a very long period of time.

Only the ground level arthropods are our research target. We considered the fact that they (especially insects), represent the dominant group (over 65% from total number of known organisms, more 80% from total number of the known animals and, probably, 99% from total existent species in nature).

We consider that, by knowing the changes induced by the pesticides action, at the level of arthropod populations, their impact upon all biocenosis components can be estimated by extrapolation.

We justify our orientation on the investigations upon ground level fauna both by the possibility of continuous sampling, using the ground level traps, and by utilisation of an attractant to ensure the collection on a large crop surface.

Quantitative and qualitative analyses can make, by species determination, species abundance and frequency estimation.

The comparison of all quantitative and qualitative parameters from the unpolluted and polluted samples is reflected in the collected material of the population changes, and in consequence, changes in its integrative system at the biocenosis level.

THE QUALITATIVE STRUCTURE OF THE COLLECTED MATERIAL

The collected arthropod species at the ground level, from unpolluted and polluted crops, belong to the following groups: *Arachnida (Aranea* and *Acarina)* and *Insecta (Orthoptera, Homoptera, Heteroptera, Neuroptera, Hymenoptera, Coleoptera* and *Diptera).*

Characteristic insect species living at the ground level were: *Coleoptera (Carabidae, Staphylinidae), Orthoptera (Gryllidae, Acrididae), Hymenoptera (Formicidae* and *Myrmicidae), Collembola.*

Coleoptera species were represented by *Carabidae (Ophonus rufipes* Deg., *Harpalus distinguendus* Duft., *Poecilus cupreus* L., *Abax ater* L.), *Staphylinidae* (undetermined), *Anthicidae (Formicomus pedestris* Rossi), *Elateridae (Agriotes lineatus* L.), *Tenebrionidae (Opatrum sabulosum* L.), *Dermestidae (Dermetes laniarius* Illig.), *Chrysomelidae (Leptinotarsa decemlineata* Say, *Phyllotreta vittula* Redt., *Ph. nemorum* L., other undetermined species), *Curculionidae (Tanymecus dilaticollis* Gyll.).

Orthoptera species were *Gryllus campestris* L., *Grylulus burdigalensis* Latr., *G. desertus* Pallas, *Dociostaurus maroccanus* L.

Hymenoptera, besides *Formicidae* and *Myrmicidae* includes also *Diapriidae (Trichopria* sp., *Loxotropa tritoma* Thoms), *Scelionidae (Telenomus chloropus* Thoms.), *Platygasteridae (Trichachis tristis* L.), *Trichogrammatidae (Trichogramma* sp.), and other undetermined Chalcidoidea.

Homoptera species were *Empoasca solani* (Curt.), *Macrosteles sexnottatus* Fall., *Macrosiphum euphorbiae* (Hott et Fris.), *Acyrthosiphon pisum* Harr, *Sitobion avenae* Fabr..

Heteroptera species were *Lygus rugulipennis* Poppius and *Eurygaster integriceps* Put.

Diptera Brachycera species were present in the samples, attracted by vinegar. There were also identified *Contarinia tritici* Kirby, some *Sciaridae*.

Crustacea-Isopoda was represented by *Armadillidium vulgare*.

INSECTS NUMBER IN THE SAMPLES FROM UNPOLLUTED AND POLLUTED CROPS

In both unpolluted and polluted samples, the insects were prevalent as species and number of individuals. In some polluted samples, only insect species were present.

The attention was drawn by the fact that in the samples collected from the polluted crops, the insects number was smaller comparatively with the similar unpolluted ones, especially in the first day after pesticide application.

In most cases, in polluted crop, the increase of the Arthropod number was determined especially by the increase of the number of *Coleoptera, Diptera* and *Aranea.* Perhaps, these groups have a bigger resistance to pesticides.

COMPARISON BETWEEN THE FAUNISTIC STRUCTURE FROM POLLUTED AND UNPOLLUTED CROP

In the **unpolluted crop** there were present some specific pests for potatoe crop, from the adjacent or precursory crops, some polyphagous and accidental species.

The specific pests for potatoe crop were *Leptinotarsa decemlineata* and *Empoasca solani.*

Polyphagous species were some *Orthoptera (Gryllus campestris, Gryllulus burdigalensis, G. desertus, Dociostaurus maroccanus), Heteroptera (Lygus rugulipennis), Coleoptera (Ophonus rufipes, Harpalus distinguendus, Opatrum sabulosum).*

Most arthropod species in the unpolluted crops were, however, **predators** *(Aranea, Coleoptera-Carabidae, Anthicidae, Staphylinidae, Coccinellidae)* and **parasitoids.**

In the **polluted crop,** the **pests species** (*Homoptera-Cicadellidae, Aphididae, Heteroptera, Thysanoptera, Coleoptera-Harpalidae, Tenebrionidae, Curculionidae* and *Diptera)* were prevalent. The **predators** were represented mostly by *Aranea,* some *Carabidae* and *Staphylinidae.* The **parasitoids** were not found.

THE INFLUENCE OF ABIOTIC FACTORS AND ADJACENT CROPS UPON FAUNISTIC STRUCTURE

In the unpolluted crop one can remark some fluctuations in the species and individuals number, as a result of reduction of food quantity and quality, decrease of humidity, and temperature increase at the soil level.

The adjacent crops (barley, sunflower, pea) were an important source for the enrichment of faunistic structure, through immigration of some specific, polyphagous (phytophagous, predators), necrophagous, detritophagous species (Table 1, 2).

Table 1

Influence of adjacent crops (barley, sunflower, pea) upon arthropod individuals number in potatoe unpolluted crop

Arthropoda	Barley	Sunflower	Pea	Total
Crustacea	—	2	—	2
Arachnida	24	17	15	56
Insecta	150	29	86	265
Total	174	48	101	323

Table 2

Influence of adjacent crops upon individuals number in potatoe polluted crop

Arthropoda	Barley	Sunflower	Pea	Total
Crustacea	—	—	—	—
Arachnida	7	9	6	22
Insecta	35	18	20	73
Total	42	27	26	95

The adjacent crops infused their specific and polyphagous pests *(Eurygaster integriceps, Sitobion avenae, Acyrthosiphon pisum, Macrosteles sexnottatus, Haplothrips tritici, Harpalidae, Opatrum sabulosum, Phyllotreta vittula, P. nemorum, Tanymecus dilaticollis, Contarinia tritici, Orthoptera* species).

There was no connection between immigrant phytophagous and parasitoid species, with potatoe crop, but the polyphagous predators play a certain role in their pest reduction.

If the adjacent crop was barley, which offered good conditions for different invertebrate species, the traps collected more arthropod species and individuals.

In the traps installed near sunflower and pea crops, the number of species and individuals were comparatively smaller, because these crops were characterised by a numerically reduced arthropod fauna.

The previous crop enriched faunistic structure, especially by the species which hibernated in the soil or in the plants remained in the field.

COMPARISON BETWEEN THE NUMBER OF SPECIES AND INDIVIDUALS, IN UNPOLLUTED AND POLLUTED CROP

In all cases, the number of species, but more significantly of individuals, in polluted potatoe crop has been lower than in the unpolluted crop (even half or more). (Tables 3–8).

Table 3

Comparison between arthropod individuals number in unpolluted potatoe crop (near barley)

Arthropoda	11	12	13	14	15	Total
Crustacea	—	—	—	—	—	—
Arachnida	1	7	3	5	8	24
Insecta	25	22	28	48	27	150
Total	26	29	31	53	35	174

Table 4

Comparison between arthropod individuals number in unpolluted potatoe crop (near sunflower)

Arthropoda	11	12	13	14	15	Total
Crustacea	—	—	1	—	1	2
Arachnida	3	3	2	4	5	17
Insecta	3	4	5	7	10	29
Total	6	7	8	11	16	48

Table 5

Comparison between arthropod individuals number in unpolluted potatoe crop (near pea)

Arthropoda	11	12	13	14	15	Total
Crustacea	—	—	—	—	—	—
Arachnida	1	—	5	3	6	15
Insecta	16	16	18	18	18	86
Total	17	16	23	21	24	101

Table 6

Comparison between arthropod individuals number in polluted potatoe crop (near barley)

Arthropoda	17	18	19	20	21	Total
Crustacea	—	—	—	—	—	—
Arachnida	1	1	—	4	1	7
Insecta	4	6	8	6	11	35
Total	5	7	8	10	12	42

Table 7

Comparison between arthropod individuals number in polluted potatoe crop (near sunflower)

Arthropoda	17	18	19	20	21	Total
Crustacea	—	—	—	—	—	—
Arachnida	1	1	2	3	2	9
Insecta	2	2	4	5	5	18
Total	3	3	6	8	7	27

Table 8

Comparison between arthropod individuals number in polluted potatoe crop (near pea)

Arthropoda	17	18	19	20	21	Total
Crustacea	—	—	—	—	—	—
Arachnida	1	2	—	1	2	9
Insecta	2	2	4	6	6	20
Total	3	4	4	7	8	26

The reduction was accentuated especially for *Insecta* species (73:265, so, over three times). The total number of the *Arthropoda* individuals was less than a quarter (95:323).

The reduction of arthropod population level was the result of the negative impact of pesticides.

If man wants pests reduction, at the same time he unintentionally destroys pest natural enemies (predators and parasites). As a result, the natural balance is disturbed and favourable conditions are created for increasing pest populations.

COMPARISON BETWEEN THE NUMERICAL ABUNDANCE IN THE UNPOLLUTED AND POLLUTED CROP

In **unpolluted crop,** the greatest abundance was displayed in most cases by *Aranea, Orthoptera, Coleoptera, Hymenoptera-Formicidae,* generally represented by species living at the ground level. Amoung *Coleoptera,* **predators** species were numerically abundant.

Due to their large size, the *Coleoptera, Orthoptera* were also gravimetrically abundant.

Parasite species had a low abundance, due to the limits of the sampling method.

In the **polluted** crops, the *Coleoptera* were also dominant, but represented especially by **phytophagous** *(Ophonus, Harpalus, Opatrum, Agriotes, Tanymecus)* and **necrophagous** *(Dermestes)* species.

A decrease of the Aranea abundance was noticed, perhaps as a result of pesticide impact.

COMPARISON BETWEEN WEIGHT OF THE DIFFERENT TROPHIC CATEGORIES

In potatoe crop the food chains were short and food web is poor.

In the **unpolluted potatoe crop,** the secondary consumers *(Carabidae, Staphylinidae, Anthicidae, Coccinellidae, Chrysopidae* predator species and *Diapriidae, Scelionidae, Platygasteridae, Trichogrammatidae,* other *Chalcidoidea* parasitoid species) were dominant, comparatively with the primary consumers *(Orthoptera, Homoptera, Thysanoptera, Heteroptera, Harpalidae, Tenebrionidae, Chrysomelidae, Curculionidae* phytophagous species).

The dominance of these natural enemies is an especially positive fact because these secondary consumers represent very important biotic factors of pests populations control, with a benefic limitation action upon pest populations, which they can maintain at lower levels.

The prevalence of the primary consumers, in the **polluted** crop, represents a good indicator of the ecological unbalance induced by the pesticide action. The primary consumers are much more resistant, making use of their chemical mechanisms with a large spectrum (characteristic for phytophagous species), for detoxification of pesticides. This phytophagous characteristic, together with the strong pesticide impact upon the secondary consumers (lack to chemical detoxification mechanisms), leads to the change of the ratio between the two trophic categories of organisms.

The pests resistance and destruction of their natural enemies by pollutants create the possibility of the effective pests increase and, in consequence, economic damages are generated.

The **pollinators** were absent both in the unpolluted and polluted crop, absence resulting from the sampling method limits, which excluded, at least partially, the anthophylous species.

The necrophagous arthropods occurred only accidentally in some unpolluted and polluted samples.

THE ARTHROPOD GROUPS FREQUENCY IN UNPOLLUTED AND POLLUTED CROP

In the samples collected from unpolluted crop, present in all samples, were *Insecta* species.

Constant Arthropoda were *Aranea, Orthoptera-Gryllidae, Hymenoptera-Formicidae and Serphidae, Coleoptera* species. (Table 9).

Accessories species were *Homoptera* and *Diptera-Barchycera.*

Accidental species was *Crustacea — Armadillidium vulgare.*

Table 9

Arthropoda frequency (%) in unpolluted and polluted samples

Arthropoda	Unpolluted	Polluted
Crustacea	40	—
Arachnida	80	100
Insecta	100	100

In the samples collected from **polluted crop, euconstant species** were Aranea, *Coleoptera* (pest species), *Hymenoptera* and **constant species** were *Homoptera* and *Diptera.*

Crustacea were not found.

THE DYNAMICS OF ARTHROPOD SPECIES AND INDIVIDUALS NUMBER

A fluctuation of the number of species and individuals could be noticed, in unpolluted crop, due to the abiotic factors variation and crop characteristic. (Table 10).

Species number varied between 12 and 18. Individuals number varied between 49, the lower value, and 87, almost double.

Table 10

Dynamics of Arthropoda species and individuals number in unpolluted potatoe crop

Quantitative indexes	11	12	13	14	15
Species number	12	17	15	14	18
Individuals number	49	52	62	87	65

After chemical application, the pesticide induced a sudden and accentuated decrease of arthropod populations. (Table 11).

Table 11

Dynamics of Arthropoda species and individuals number in polluted potatoe crop

Quantitative indexes	17	18	19	20	21
Species number	6	8	11	11	12
Individuals number	11	14	18	25	27

The next days, a gradual faunistic reinstallation took place from adjacent crops. Species number varied between 6 and 12, but individuals number was smaller, this time, with values only between 11 and 27.

CONCLUSIONS

Human intervention by chemical control of potatoe pest populations was responsible for changes in food web; the negative consequences at the individual and populational level were detected at the entire *Arthropoda* community, too.

The pesticide affected all categories of organisms, species interrelations, perturbing the predator-prey, parasitoid-host, phytophagous-plant, systems.

These perturbations manifested as changes in species abundance, frequency, dominance and constancy, as decrease of food web complexity. The biocenosis diversity and, in consequence, their stability, decreased.

Very affected were natural enemies populations (parasitoids, predators); this fact had as the result the increase of pest populations (specific and polyphagous).

In sustainable agriculture, an alternative for exclusive use of pesticides is the implementation of integrated pest management, with emphasis on maximizing the natural control efficiency and nonchemical tactics (biological, cultural, physical and mechanical control methods, utilisation of plants resistance). Chemical control must be limited in space and time, for preserving the natural enemies in the field, to maintain pest populations at economic acceptable levels.

The negative effects of pesticides must be diminished essentially by development-conservation conception change, by making protection at the basis of development.

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John C. Briggs. *Global Biogeography* (Developments in Palaeontology and Stratigraphy, *14*), Elsevier, Amsterdam, 1995; XVII + 454 p.

The author is a well-known ichthyologist and zoogeographer who published numerous contributions in both fields u. oth. the monograph of the fish order Xenopterygii (1955), a Marine Zoogeography (1974), Centres of origin in biogeography, (1984) Biogeography and plate tectonics (1987), besides tens of contributions in journals, etc. He is one of the leaders of the School of dispersalist biogeography.

The first chapter of the book deals with the history of biogeography from the 17-th century up the late 80-ties. Special subchapters are devoted to the advent of continental drift, reactualized as "plate tectonics" and to the rise of vicariism (and of panbiogeography). The author is fully justified when asserting that the School of vicariance biogeography "asks the investigators to perform an act of faith before he begins his analysis; he must believe in a given first-order explanation". Actually, the distribution of any taxon is the result of range extension and fragmentations (vicariante events) followed by geographic specifications and extinctions.

The bulk of the book (p. 19 to 189) consists of chapters 2 to 7 grouped in the section "Historical biogeography". The configurations of the continents and oceans during ten geological periods (from the Precambrian to the Pleistocene) and the distribution of fauna and flora is described, according to most recent geological, paleoclimatological and paleontological data; the text includes a high number of maps. Only few pages are devoted to the earlier periods (Precambrian to Jurassic), much more to the Cretaceous, to the three periods of the Paleogene and especially to the Neogene and Pleistocene. Marine provinces alone could be delimited prior to the Carboniferous; starting from the carboniferous also terrestrial ones. Data on the freshwater fauna and its regional distribution and dispersal are mentioned for the Jurassic, more detailed ones for the Cretaceous, the Noegene and the Pleistocene.

Numerous concrete cases of dispersals between continental and marine areas, determined by geographical modifications, are mentioned. Each chapter of this historical section is followed by a summary. Extinctions which occurred during all periods, e. g. at the limits between Ortovician and Silurian, Devonian and Carboniferous, end Permian, late Triassic, etc. are also mentioned. Remarkable are data on the extension of the Tethys and limits of its regional subdivisions during the Cretaceous (Fig. 33); these are still reflected in the present-day distribution of the marine littoral fauna and especially in that of the hypogean lineages of peracarid crustaceans in inland waters. The historical section of the book also includes a last chapter (8) devoted to extinctions, their presumed causes and consequences.

A second extended section of the book, "Contemporary biogeography", comprises two chapters (9 and 10) devoted to the marine patterns, one (11) to the terrestrial (and freshwater) patterns, one to "significant patterns", one to species diversity and a short "epilogue".

The tropical marine regions are dealt with in chapter 9, the temperate and cold littoral ones, besides the pelagic and the benthic realms, in chapter 10. The author considers that the

Indonesian-Malayan area has been the center origin and dispersal from which the other Indo-West Pacific areas have been populated; within most lineages, the apomorphic species occurs in this center, the archaic (plesiomorphic) ones inhabiting the peripheral areas (a situation identical with that in most terrestrial and freshwater lineages, as pointed out mainly by Darlington). The role of deep water and terrestrial barriers in maintaining isolated the tropical shelf regions is emphasized. Data on the deep benthic fauna are based to a large extent on quite recent contributions.

About 18 pages, including numerous maps, are devoted to the freshwater fauna. Most data refer to primary and secondary freshwater fishes, only few peripheral families being mentioned. Crayfishes, unionacean mussels, bathynellanceans, aquatic earth worms are also dealt with, besides several lineages of aquatic insects, five of which have not been analyzed in Banarescu's 1990–1995 Zoogeography of freshwaters.

Dealing with terrestrial habitats, Briggs analyzes the distribution and probable dispersal history of a few families of insects, spiders and scorpions, of birds, mammals, reptiles and amphibians of several groups of plants (from bryophytes to angiosperms).

Much attention is given to the fossil representatives of all continental lineages, A 7-pages summary of the main distribution patterns and dispersal history of the continental lineages points out the reality of the zoogeographic regions delimited by Sclater and Wallace more than a century ago, the fact that Madagascar, Australia and New Zealand have no more been connected to the other continents since the Upper Jurassic, that each lineage had its own center of origin, their later dispersal history depending on the evolution of continents (for example the direct connections between Europe and North America ceased in the Eocene, Europe and Siberia came in contact during the Oligocene, etc.).

Two "significant" distribution patterns are dealt with in Chapt. 12: the antitropical distributions (most examples mentioned are from the marine fauna, some from the terrestrial one) and island life. Many explanations of antitropical distributions have been advanced; the author discusses them, concluding that the majority of these distributions result from extinctions in this tropical zone. The section devoted to island life considers mainly oceanic islands and a few remnants of continental crust (Darlington's "fringing archipelagos"). The degree of endemism is considered to depend on the age of the island; the animals and plants to have colonized the island across the sea, the species flocks to have originated through repeated phenomena of double colonizations. Mac Arthur's and Wilson's theory of island biogeography is not mentioned.

A last chapter is devoted to biodiversity: this is much higher in terrestrial habitats than in marine ones. The Indo-West Pacific shelf fauna has the richest marine fauna. Biodiversity is much higher at lower than at higher latitudes, both in the sea and on continents.

The references include 32 pages; many titles mentioned in the text have been omitted from the references; among these some are in German or French. Remarkable is the high number of references published during the 90-ties. It is regretable however that two important books have been omitted: Jeannel's "Genèse des faunes terrestres" (1942) which explains the dispersal history of numerous lineages, mainly of insects, in the light of continental drift, and Theniu's two editions (1972 and 1980) of "Grundzüge der Faunen — und Verbreitungsgeschichte der Säugetiere", the most detailed overview of the dispersal history of mammals that has been published.

Biogeographers must enjoy that such a book that uses numerous palogeographic and paleontological data on a high number of lineages has been elaborated in a period when many authors try to explain dispersals of whole biota in the base of few (and very significant) lineages, including but a limited number of species.

Vicarianists and pan-biogeographers assert that they alone correlate evolution of life with that of continents. Actually, many dispersalists, (not only Briggs, but also Darlington and others) make this correlation, even in a better manner, since they take into consideration the fossils. Accepting that each lineage has its own initial dispersal centre, dispersalists explain the later evolution and dispersal history of the lineages on the base of known paleogeographic data, events such as the establishment of the Central American link having favoured the dispersal of many lineages and species, often of different origin.

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NOTE POUR COLLABORATEURS

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 somaires des revues sont complétés 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.

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La responsabilité pour le contenu des articles revient exclusivement aux auteurs.

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