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CEROXYS BANEAI, NOUVELLE ESPÈCE D'OTITIDAE (DIPTERA) DE ROUMANIE

V. GHEORGHIU

A new species of *Otitidae*, *Ceroxys baneai*, is described and illustrated. The new species is compared with *C. urticae* L.

INTRODUCTION

Deux espèces de *Ceroxys* ont été signalées jusqu'ici dans notre pays : *hortulana* Rossi (10, 11 et 5) et *urticae* L. (5).

L'examen de genitalia des 361 individus d'*Otitidae* provenant de différents départements (tableau 1), supposés apparttenir à *C. urticae* et recueillis par l'auteur, ou mis à notre disposition par les chercheurs suivants : Alin Constantin, **A.C.** (dans le texte) ; Aurelian Popescu-Gorj, **A.P.G.** ; Atena Roşca, **A.R.** ; Ionel Andriescu, **I.A.** ; Dumitru Sofronie, **D.S.** ; Maria Cantoreanu, **M.C.** ; Maria Iacob, **M.I.** ; Medeea Weinberg, **M.W.** ; Vladimir Brădescu, **V.B.** ; Xenia Scobiola-Palade, **X.S.P.**, nous a permis de découvrir une nouvelle espèce, *C. baneai*. En outre, nous avons trouvé dans ce matériel quelques exemplaires de *C. munda* Loew, signalé ici pour la première fois dans le pays.

Le genre *Ceroxys*, avec une répartition paléarctique, est connu donc à présent en Roumanie par quatre espèces : *C. hortulana* Rossi, *C. urticae* L., *C. munda* Loew et *C. baneai* n. sp..

Tableau 1

Stations de récolte et leur localisation dans le système U.T.M. (dressé par 3)

No.	Localité	Departement	U.T.M.	Code
1.	Agigea	Constanţa	PJ	28
2.	Băile Herculane	Caraş—Severin	FQ	17
3.	Bucureşti	—	MK	21/22
4.	Budeşti	Giurgiu	MJ	51
5.	Caraorman	Tulcea	PK	89
6.	C. A. Rosetti	Tulcea	QL	01/02
7.	Dăbuleni	Dolj	KJ	65
8.	Halmeu Vii	Satu Mare	FU	51
9.	Măcin	Tulcea	NL	81
10.	Măgura	Constanţa	NJ	85
11.	Mehadia	Caraş-Severin	FQ	07
12.	Pădurea Răioasa	Ilfov	MK	12
13.	Slănic Moldova	Bacău	MM	51
14.	Sulina	Tulcea	QL	00
15.	Svinița	Mehedinţi	EQ	83
16.	Turulung	Satu-Mare	FU	51
17.	Valea Cernei	Caraş-Severin	FQ	16

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Ceroxys bancai n. sp.

Localité type : Mehadia, 10 juliet 1954, leg. Atena Rosca.

MATÉRIEL ET MÉTHODE

Holotype : 1. mâle, Mehadia — Caraş Severin, le 10 juillet 1954, leg. **A.R.** ; *allotype* : 1. femelle, Caraorman — Tulcea, 12 juillet 1984 ; *paratypes* : 1 ♂, **1** (dans le tableau), 18.VII.1962, **X.S.P.** ; 1 ♀, **15**, 19. VII.1962, **A.P.G.** ; 1 ♂, **6**, 30.VI.1971, **M.W.** ; 3 ♂♂, **6**, 26.V.1971, **V.B.** ; 1 ♀, **15**, 31.V.1971, **A.P.G.** ; 1 ♂, **6**, 31.V.1972, **A.C.** ; 1 ♂, **3**, 26.VI.1971, **M.I.** ; 1 ♂, **6**, 4.VI.1981, **V.B.** ; 1 ♀, **6**, 1.VII.1983, **V.B.** ; 4 ♂♂ 1 ♀, **5**, 7.VIII.1984, ; 1 ♀, **5**, 17.VII.1985 ; 1 ♂ 1 ♀, **5**, 18.VII.1985 ; 3 ♂♂, **17**, 10.VI.1986, **I.A.**

C. urticae L. : 1 ♀, **1**, 22.VI.1962, **V.B.** ; 1 ♂, **1**, 31.V.1962, **A.P.G.** ; 1 ♀, **12**, 14.VII.1962, **M.W.** ; 1 ♂, **1**, 27 — 29.V.1963, **A.P.G.** ; 1 ♂, **5**, 12.VI.1967, **D.S.** ; 1 ♀, **7**, 17.VIII.1967, **M.C.** ; 1 ♀, **15**, 7.VI.1968, **M.W.** ; 1 ♂, **5**, 15.V.1968, **X.S.P.** ; 1 ♀, **15**, 17.VI.1968, **M.W.** ; 1 ♂ 4 ♀♀, **5**, 11.VI.1962, **M.W.** ; 1 ♀, **15**, 16.VI.1969, **M.W.** ; 1 ♀, **15**, 17.VI. 1969, **M.W.** ; 1 ♂ 1 ♀, **15**, 16.VII.1969, **A.P.G.** ; 1 ♀, **15**, 19.VII.1970, **A.P.G.** ; 3 ♂♂ 4 ♀♀, **6**, 26.V.1971, **V.B.** ; 1 ♀, **3**, 26.VI.1971, **M.I.** ; 2 ♂♂, **6**, 4.VII.1971, **M.W.** ; 1 ♀, **5**, 11.VIII.1971, **X.S.P.** ; 1 ♂ 1 ♀, **6**, 9.VII. 1972, **M.C.** ; 4 ♂♂, **6**, VII.1972, **A.P.G.** ; 4 ♂♂ 1 ♀, **13**, 9.VII.1972, **M.W.** ; 1 ♂, **10**, 26.VI.1973, **A.P.G.** ; 2 ♂♂, **15**, 4.VI.1979, **V.B.** ; 3 ♂♂ 1 ♀, **6**, 7.VI.1979, **V.B.** ; 1 ♂, **5**, 22.VII.1979, **A.P.G.** ; 1 ♂, **6**, 15.VII.1980, **X.S.P.** ; 1 ♀, **6**, 7.IX.1980, **X.S.P.** ; 1 ♂, **15**, 4.VI.1981, **V.B.** ; 1 ♂, **6**, 5.VI.1981, **V.B.** ; 1 ♀, **6**, 6.VI.1981, **V.B.** ; 1 ♂, **2**, 4.IX.1982, **I.A.** ; 1 ♀, **6**, 10.X.1982, **X.S.P.** ; 2 ♀♀, **6**, 3.VII.1983, **V.B.** ; 1 ♂, **6**, 1.VIII.1983, **V.B.** ; 2 ♂♂, **6**, 29.VII.1984, **V.B.** ; 1 ♂ 1 ♀, **5**, 17.VI.1984, **V.B.** ; 1 ♂ 2 ♀♀, **5**, 19.VI. 1984, **V.B.** ; 1 ♀, **17**, 29.VI.1984, **I.A.** ; 10 ♂♂ 7 ♀♀, **5**, 7.VIII.1984 ; 1 ♂, **5**, 4.VI.1985, **I.A.** ; 1 ♂, **17**, 15.VI.1985, **I.A.** ; 7 ♂♂ 5 ♀♀, **17**, 27.VI. 1985, **I.A.** ; 1 ♂, **5**, 18.VII.1985 ; 3 ♀♀, **5**, 20.VII.1985 ; 1 ♀, **5**, 21.VII. 1985 ; 7 ♂♂ 9 ♀♀, **17**, 10.VI.1986, **I.A.** ; 10 ♂♂ 7 ♀♀, **17**, 11.VI.1986, **I.A.** ; 1 ♂, **8**, 21.VII. 1987 ; **9**, **10**, **14**, **16** — (5).

Quant à la méthode d'étude, nous précisons que pour avoir les meilleurs résultats les genitalia doivent être designés dans une position parfaitement horizontale et les sternites VI — VIII détendus. Matériel dans notre collection.

DESCRIPTION

Tête. Toutes épines noires : 1 *vti*, 1 *vte*, 1 *vpt*, 1 *oc*, 2 *ors*. Couleur de fond : jaune, jaune-roux. Bande frontale d'un jaune-roux et pourvue de quelques petites épines dans la moitié antérieure, devient rougeâtre dans la moitié postérieure et se rétrécit graduellement, continuant de même dans le reste de la partie postérieure de la tête, jusqu'au cou. De chaque côté de la bande frontale, l'espace interoculaire est rougeâtre dans ses deux tiers distales, pour devenir noir dans le tiers proximal, s'élargissant en même temps graduellement. La bande noire continue dans la partie postérieure de la tête où elle occupe toute la surface jusqu'à la zone du péristome, s'arrêtant à l'épine peristomale. L'espace intraoculaire présent dans cette zone une pruinosité gris-argentée, qui commence de la moitié du front et s'étend sur toute la couleur noire jusqu'à la moitié su-

périeure de la partie postérieure de la tête. La moitié inférieure de la partie postérieure de la tête est d'un noir luisant, passant graduellement en jaune-roux. Sur la partie dorsale de la tête on voit deux lobes noirs séparés par une mince bande rougeâtre ; chez *C. urticae* la partie postérieure de

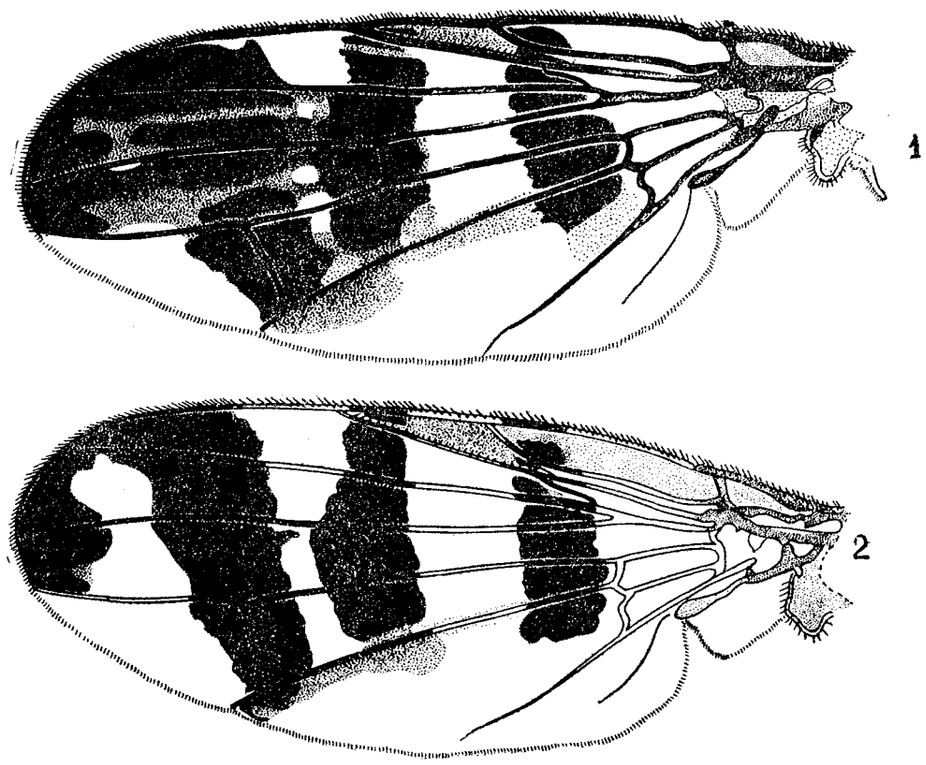

Fig. 1. — Aile *Ceroxys baneai* n. sp.
Fig. 2. — Aile *Ceroxys urticae* L.

la tête est complètement jaune-rougeâtre dans sa moitié inférieure. Le triangle occellaire est noir et les occelles jaunes-blanchâtres. Le profil de la face est convexe, tandis que chez *C. urticae* elle est droite. La face, la carène faciale, les gènes et le péristome sont jaunes et le fond des cavités anténnaires est rougeâtre ou olive — en fonction du degré de mélanisme de l'individu. Le proboscis, rouge-pourpre ; palpes, jaunes. Le 3e article de l'antenne, jaune-rougeâtre à sommet noirâtre ; l'arista, noire au moins dans la partie basale, est couverte d'un tomentum blanchâtre.

Thorax. Poils noirs. Chétotaxie semblable à celle de *C. urticae.* Mésonotum noir, couvert d'un tomentum argenté à reflets bleu pétrole. Scutellum noir luisant, parfois olive. Pleure et coxes noires, couvertes d'une pruinosité argenté-pâle. Fémur et tibia noirs luisants. Tarses d'un jaune-rougeâtre allant jusqu'à olive — en fonction du degré de mélanisme.

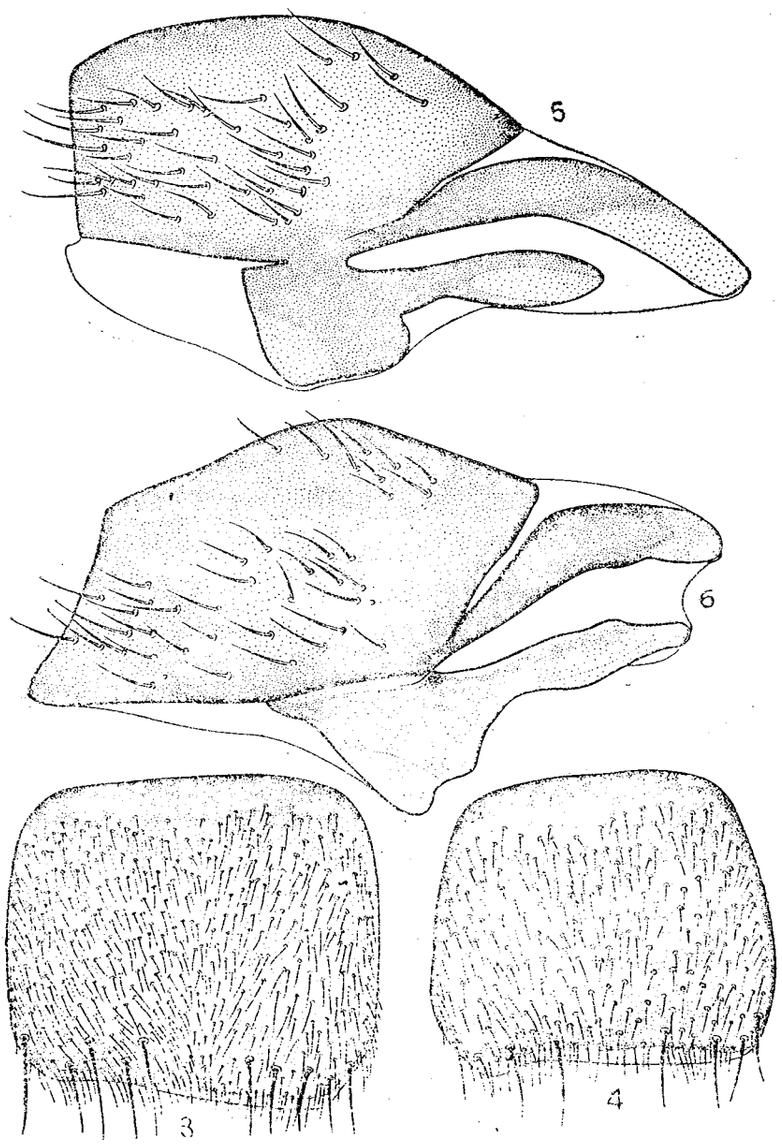

Fig. 3. — Sternite V, *Ceroxys bancai* n. sp.
Fig. 4 — Sternite V, *Ceroxys urticae* L.
Fig. 5 — Sternites VI — VIII, *Ceroxys bancai* n. sp.
Fig. 6 — Sternites VI — VIII, *Ceroxys urticae* L.

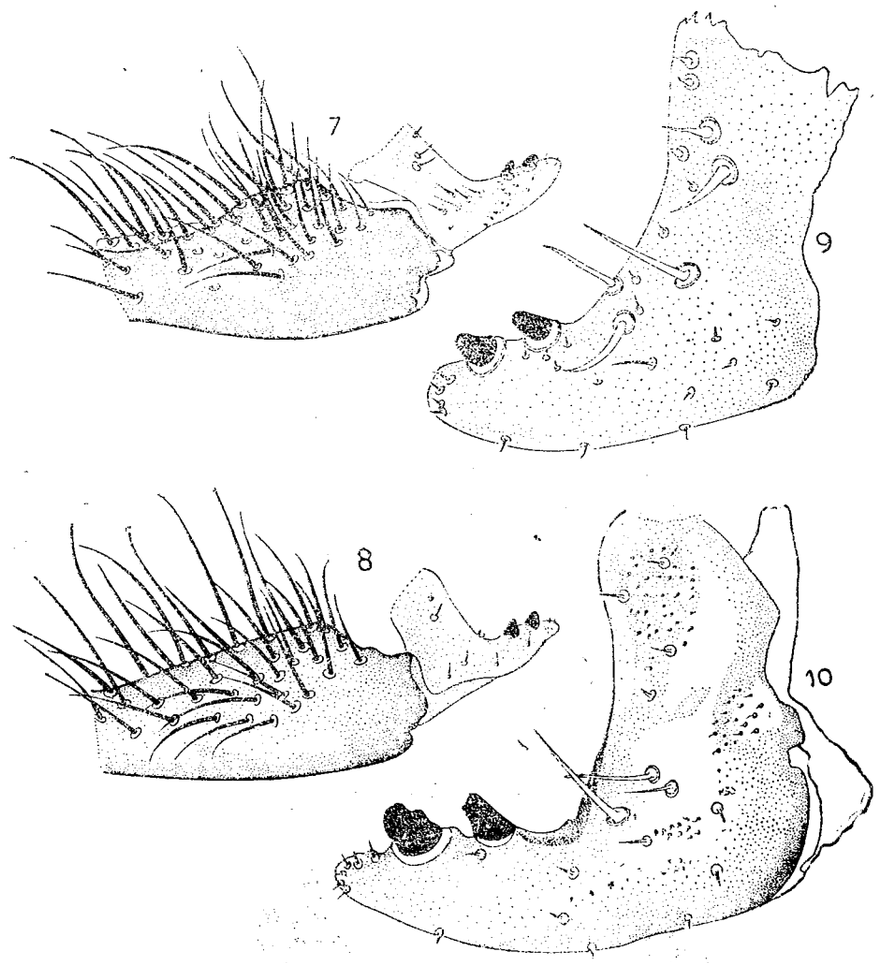

Fig. 7 — Hypopygium, *Ceroxys bancai* n. sp.
Fig. 8 — Hypopygium, *Ceroxys urticae* L.
Fig. 9 — Surstylus, *Ceroxys bancai* n. sp.
Fig. 10 — Surstylus, *Ceroxys urticae* L.

Aile. Les taches de couleur sombre, qui partent du sommet des cellules *R1* et *Sc*, ont tendance à s'unir; parfois elles s'unissent le long de la nervure *CuA1* formant avec la tache sombre qui part du sommet de la cellule 2*C* un « W ». La couleur dominante est jaune. Chez *C. urticae* le blanc prédomine entre les bandes sombres nettement séparées (figs. 1, 2). En tenant compte de la coloration des ailes et du IV-e tergite, nous avons dressé une clé de détermination pour les quatre espèces de *Ceroxys* trouvées jusqu'à présent en Roumanie

1. Aile dépourvue de bandes sur *ta* et *tp*. Bande brune étroite, partie de Cu_2, passe sur la bifurcation de la r_1 et se prolonge jusqu'à la costale qui a une tache à l'endroit des r_1, r_3, r_5 . . . . . *C. munda* Loew *
— Aile avec des bandes brunes sur *ta* et *tp* 2
2. Bande brune de *tp* est étroite et séparée de la tache brune préapicale (trouvée dans l'angle formé par la costale et la r_3) . . . . . *C. hortulana* Rossi

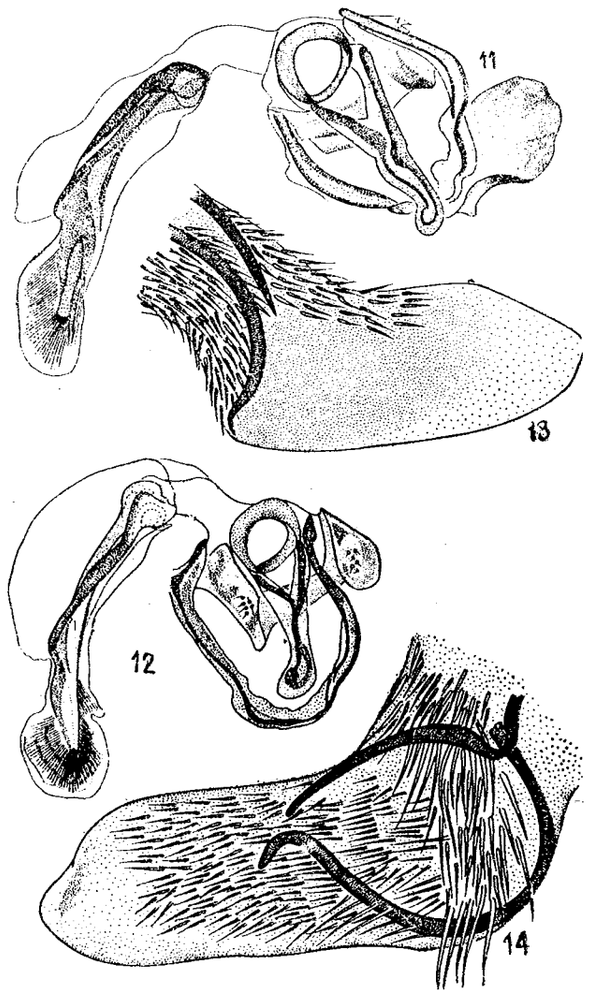

Fig. 11 — Apodème, *Ceroxys baneai* n. sp.
Fig. 12 — Apodème, *Ceroxys urticae* L.
Fig. 13 — Apex édéage, *Ceroxys baneai* n. sp.
Fig. 14 — Apex édéage, *Ceroxys urticae* L.

— Bande brune de *tp* est plus large et s'unie avec la tache brune préapicale 3
3. Tache brune du sommet de l'aile s'unit avec la bande brune large de *tp*, par l'intermède de la *R1*. Tergite IV avec deux taches argentées sur sa marge antérieure . . . . . *C. urticae* L.
— Tache brune du sommet de l'aile s'unit avec la bande brune large de *tp*, par l'intermède des *R1*, *R3* et *R5*. Les deux taches argentées de la partie anterieure du IV^e^ tergite sont unies et forment une bande . . . . . *C. baneai* n. sp.

Abdomen (figs. 3, 4). La couleur de fond est le noir luisant. Les deux taches tomenteuses argentées présents sur le IV^e^ tergite chez *C. urticae*, apparaissent chez *C. baneai* comme une bande continue de largeur plus ou moins constante. La tache jaune qui couvre les genitalia mâles est présente sur la partie apicale du V^e^ tergite.

Genitalia de mâle. La position des genitalia est le principal caractère qui différencie la nouvelle espèce de *C. urticae*. Parce que chez celle-ci l'axe de symétrie des genitalia est celui du corps, chez *C. baneai* n. sp. l'axe de symétrie est déplacé vers la gauche avec 45° — 50° (par rapport à la verticale). Ce déplacement à entraîné la modification de la forme des sternites VI — VIII (fig. 5), à l'égard de *C. urticae* (fig. 6). D'autres différences évidentes apparaissent dans la structure du sommet de l'édéage : chez *C. urticae* celui-ci est couvert d'épines (fig. 14), tandis que chez l'espèce nouvelle cette partie est nue (fig. 13). La structure de résistance diffère aussi : le surstyle du hypopyge est plus étroit et a le sommet plus pointu chez *C. urticae* par rapport à *C. baneai* n. sp. (figs. 9, 10). La structure de l'anneau copulateur est aussi différente, ainsi que celle de l'apodème éjaculateur (figs. 11, 12) qui, chez *C. urticae* ressemble à celui illustré par (1).

Genitalia de la femelle. L'ovipositeur a une structure moins complexe; il n'y apparaissent pas de différences importantes entre les deux espèces. Une étude des genitalia des quatre espèces *d'Otitidae* de Roumanie sera publiée ulterieurement.

HABITAT

L'espèce à été recontrée sur la végétation herbacée, sur des arbustes et les cannaies des zones marécageuses. Elle est attirée par la lumière artificielle.

DERIVATIO NOMINIS

En signe de profond hommage, je dédie cette espèce à mon regretté ami, le héros Florin Banea, assassiné pendant la nuit du 25 décembre 1989.

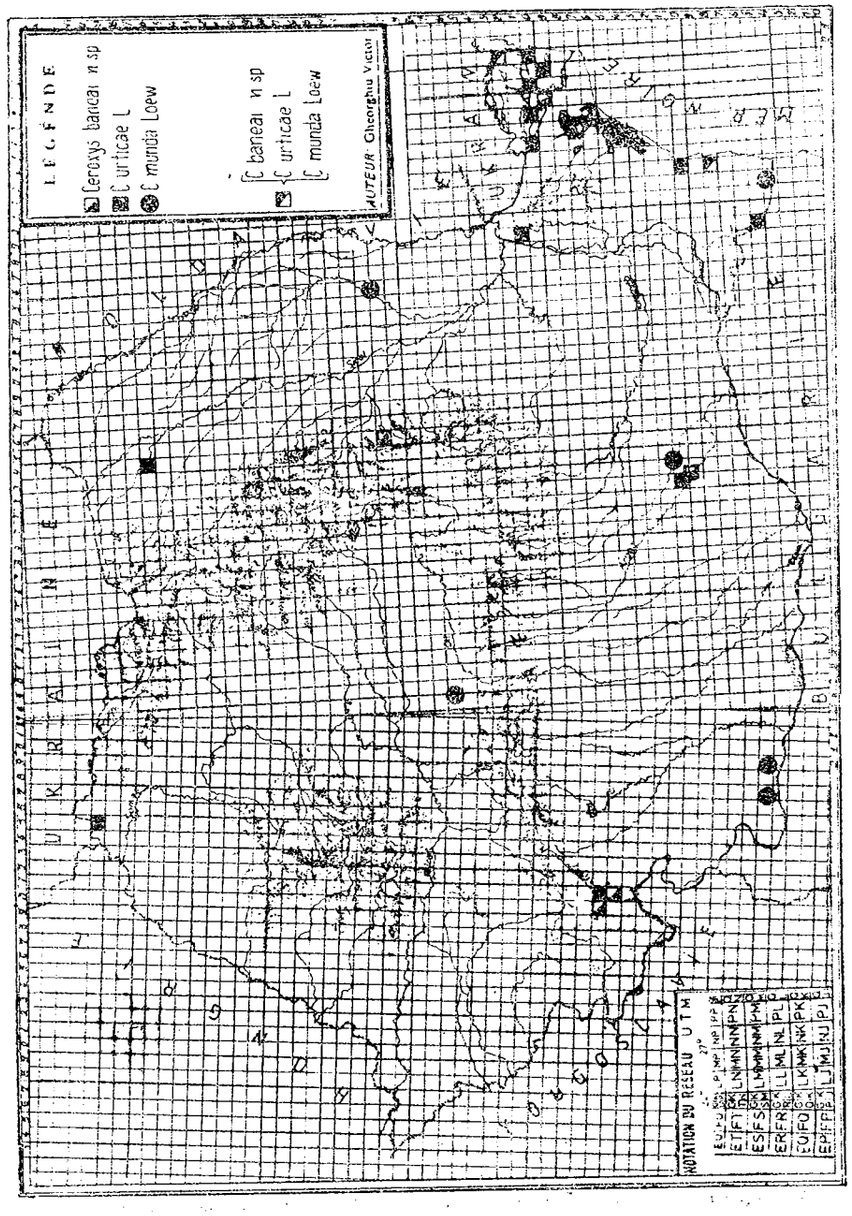

Fig. 15 — La répartition des trois espèces de *Ceroxys* en Roumanie (système, U.T.M.)

REPARTITION GEOGRAPHIQUE

Tenant compte de la distribution de *C. bancai* en Roumanie et de la valeur moyenne des températures annuelles constatées pour les endroits de capture, il est possible que cette espèce occupe la partie méridionale de l'aréal de *C. urticae* connu jusqu'à présent : l'Europe, jusqu'à la parallele de 60° (2, 4, 6, 7, 8, 9, 10). Pour la Roumanie (fig. 15).

* *Ceroxys munda* Loew. *Espèce nouvelle pour la faune de Roumanie.* Repartition géographique — (fig. 15) et (9).

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Institut de Spéologie « Emile Racovitza »
Bucarest, str. Frumoasă 11

DEUX NOUVEAUX GENRES PALÉARCTIQUES DE PARASARCOPHAGES ET LA RÉHABILITATION DU GENRE *VARIROSELLEA* HSUE (DIPTERA, SARCOPHAGIDAE)

A. Z. LEHRER

On the basis of the structural type of the male genital armature, the sub-units of the genus *Parasarcophaga* sensu Rohdendorf (nec. Johnston & Tiegs, 1921) are advanced to rank of genera. Two new genera (*Occultophalla* gen. n. with type-species: *Parasarcophaga emdeni*, Rohdendorf, 1970 and *Macabiella* gen. n. with type-species: *Parasarcophaga paularnaudi* Lehrer, 1981) are described and the genus *Varirosellea* Hsue, 1979 is rehabilitated. A key for the identification of the most frequent palaearctic genera of *Parasarcophaga* (s. lat.) is given.

Le genre *Parasarcophaga* sensu Auctoribus (nec. Johnston & Tiegs 1921) contient un très grand nombre d'espèces hétérogènes au point de vue de la typologie des armatures génitales mâles. Même sa division en 15 sous-genres, effectuée par B. B. Rohdendorf (1937, 1965) et adoptée d'une manière non-critique par tous les spécialistes contemporaines, est artificielle et pas correctement justifiée. Les différences morphologiques des caractères taxonomiques de leurs génitalies sont si grandes et si éloignées du type du „sous-genre" *Parasarcophaga* Johnston & Tiegs, que chaque sous-genre définit une unité générique bien distincte.

L'explication d'une telle conception confuse consiste dans l'étude superficielle de la morphologie de l'armature génitale et, surtout, du distiphallus de ce groupe de Sarcophaginae, dans la présentation inadéquate de ses structures et dans l'incompréhension suffisante de la valeur du plus important complexe des caractères morpho-phylogénétiques de ceux-ci.

Analysant les structures des armatures génitales mâles de tous les espèces qui se trouvent en Roumanie et de quelques espèces paléarctiques connues par nous du genre *Parasarcophaga* (s. lat.), nous avons observé que chacun de ses sous-genres est caractérisé par un type phallosomique propre et que certaines espèces sont mentenues irraisonnablement dans les sous-genres du Rohdendorf. D'autre côté, ces dernières espèces sont définies par les éléments distiphalliques très differents et très éloignés de ceux les autres congénériques, qu'elles se détachent évidemment comme espèces-types de quelques unités taxonomiques nouvelles ou incomprises par l'auteur du catalogue des Sarcophagides paléarctiques (Verves, 1986).

Ainsi, sur la base de nos principes typologiques, nous avons élevé au rang de genre tous les sous-genres de *Parasarcophaga* sensu Rohdendorf, nous avons ajouté encore deux nouveaux genres (*Occultophalla* gen. n. et *Macabiella* gen. n.) et réhabilité le genre *Varirosellea* Hsue, 1979, qui a été erronément mis en synonymie du genre *Rosellea* Rohdendorf, 1937 (supra).

REV. ROUM. BIOL. – BIOL. ANIM., TOME 39, Nº 1, P. 13–18, BUCAREST, 1994

DIAGNOSES

Genre Occultophalla gen. n.

Parasarcophaga (Liosarcophaga) sensu Rohdendorf, 1937 : Faune de l'URSS, 19(1) :204 (partim).
Parasarcophaga (Liosarcophaga) sensu Verves, 1986 : Catalogue of palaearctic Diptera, 12 :166 (partim).

Espèce-type : Parasarcophaga emdeni Rohdendorf, 1970 : Opred. nasek, evrop. časti. SSSR, 5(2) :662 (nom. n. pro *teretirostris* sensu Rohdendorf, 1937).

Diagnose. dc = 4–5 + 4–5 ; st = 1 :1 :1. Les propleures sont glabres. La cellule R_5 est ouverte. Tergite III abdominal n'a pas de macrochètes médio-marginaux. Tergite anal noir. Le distiphallus (fig. 1. A) présente seulement une seule paire de lobes membranaux longs, assez larges et plus ou moins transparents. La partie apicale articulée du paraphallus a une pièce médiane développée, longue, large, de forme irrégulière et une paire d'apophyses latérales minces, pas bifides et arrondies aux bouts.

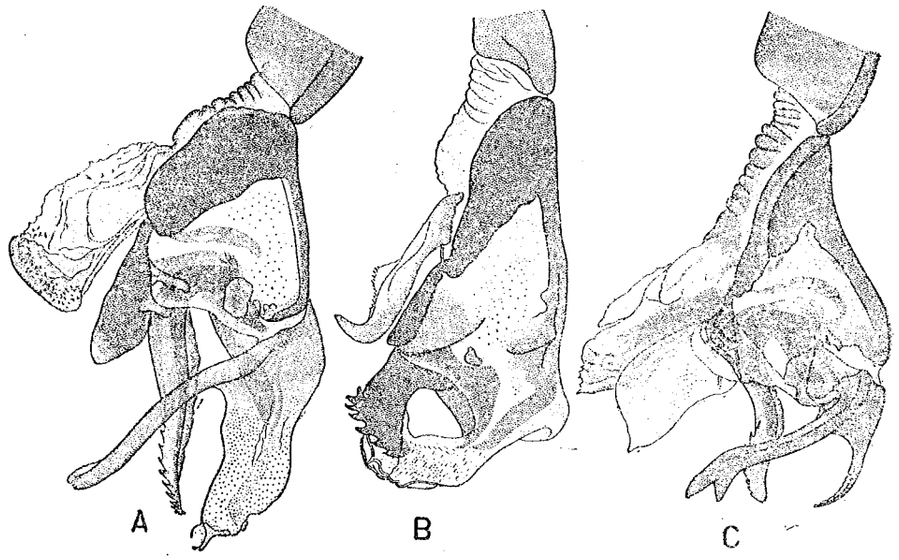

Fig. 1. — Types distiphalliques des genres **Occultophalla** gen. n., **Macabiella** gen. n. et **Liosarcophaga** Enderlein. A = *Occultophalla emdeni* (Rohdendorf) ; B = *Macabiella paularnaudi* (Lehrer) ; C = *Liosarcophaga tuberosa* (Pandellé).

Remarques. L'espèce *Parasarcophaga emdeni* Rohdendorf a été mentenue jusqu'à présent dans le "sous-genre" *Liosarcophaga* Enderlein, qui a un type phallosomique différent. Le distiphallus (fig. 1, C) est pourvu

de deux paires de lobes membranaux. La partie apicale du paraphallus a une pièce médiane courte, en forme d'un sommet aigu et courbé, et une paire d'apophyses latérales d'habitude bifides aux bouts (rarement elles ont le bout arrondi ou aigu et pourvues d'une dent subterminale).

Genre Macabiella gen. n.

Parasarcophaga (Rosellea) sensu Verves, 1986 : Catalogue of palaearctic Diptera, 12 :172 (partim).

Espèce-type : Parasarcophaga paularnaudi Lehrer, 1981 : Bull. Ann. Soc. r. belge Ent., 117 :185.

Diagnose. dc = 5 + 4 ; st = 1 :1. La cellule R_5 est ouverte. Tergite III abdominal n'a pas de macrochètes médio-marginaux. Tergite anal noir. Le distiphallus (fig. 1, B) a les lobes membranaux soudés dans une seule plaque large, avec deux sommets apicaux courbés et très pigmentés. La partie apicale du paraphallus n'est pas articulée de la partie basale et est représentée seulement par une paire d'apophyses latérales peu sclérifiées, longues et larges ; la pièce médiane manque. Les styles sont très gros, tubulaires et pourvus de dents marginales fortes.

Remarques. Parasarcophaga paularnaudi Lehrer a été introduite par Verves (1986 :172) dans le "sous-genre" *Rosellea* Rohdendorf, avec les espèces *aratrix* Pandellé et *uliginosa* Kramer.

Genre Varirosellea Hsue, 1979

Varirosellea Hsue, 1979 : Acta ent. sin., 22(2) :192.
Parasarcophaga (Rosellea) sensu Rohdendorf, 1937 : Faune de l'URSS, 19(1) :242 (partim).
Parasarcophaga (Rosellea) sensu Verves, 1986 : Catalogue of palaearctic Diptera, 12 :172 (partim).

Espèce-type : Sarcophaga uliginosa Kramer, 1908 : Ent. Wbl., 25 :152.

Remarques. D'après nos recherches, le genre *Rosellea* Rohdendorf, 1937 (avec l'espèce-type : *Sarcophaga aratrix* Pandellé, 1896) a deux paires de lobes membranaux libres (fig. 2, B) ; les styles sont lamelliformes, longs, larges, dirigés obliquement en avant et dépourvus totalement de dents récurrentes. Néanmoins, *Sarcophaga uliginosa* Kramer, qui possède aussi deux paires de lobes membranaux libres (fig. 2, A), présente les styles tubulaires, minces, longs, courbés en bas et pourvus d'un rang de dents fortes, longues et courbées sur la moitié apicale. Ces caractères génériques très importants imposent à considerer *Sarcophaga uliginosa* Kramer comme l'espèce-type du genre valid *Varirosellea* Hsue, 1979, qui a été erronément synonymisé par Verves (1986 :163).

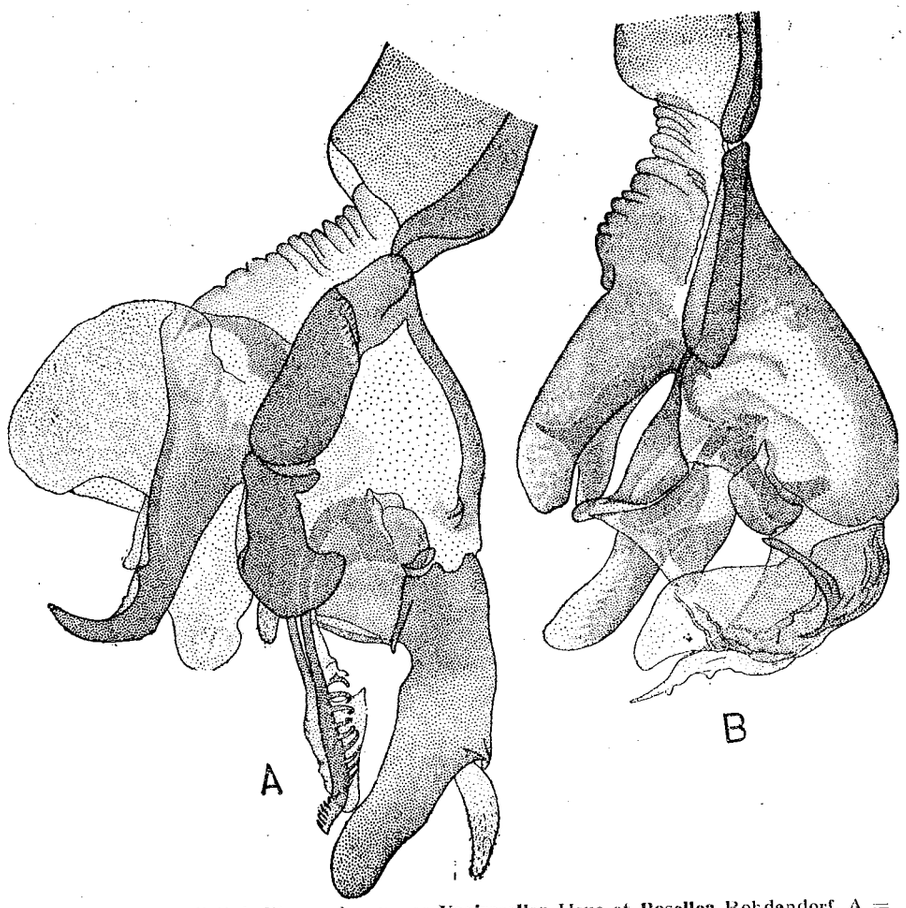

Fig. 2. — Types distiphalliques des genres **Varirosellea** Hsue et **Rosellea** Rohdendorf. A = *Varirosellea uliginosa* (Kramer); B = *Rosellea aratrix* (Pandellé).

CLÉS DES GENRES

Pour identifier et caractériser d'une manière synthétique les plus fréquents genres euroasiatiques de Parasarcophages, nous présentons les clés suivantes :

1 (4) Le distiphallus a les lobes membranaux impaire 2
2 (3) st = 1 :1 :1. Les lobes membranaux ont la forme plus ou moins d'une fleur à pédoncule. Les styles sont très courts et minces, pourvus de dents récurrentes microscopiques . . . . . .**Parasarcophaga** Johnston & Tiegs
3 (2) st = 1 :1. Les lobes membranaux sont soudés dans une plaque à deux sommets courbés en forme de crochets. Les styles sont très gros et pourvus de grosses dents récurrentes (fig. 1, B). . . . . .**Macabiella** gen. n.
4 (1) Les lobes membranaux sont paires et sans pédoncules . . . 5
5 (6) La partie apicale du paraphallus a une pièce médiane rélativement petite sous la forme d'un sommet aigu et deux apophyses latérales d'habitude bifides aux bouts ou avec une dent subterminale (fig. 1, C) . . . . . **Liosarcophaga** Enderlein
6 (5) La partie apicale du paraphallus est autrement construite ; les apophyses latérales sont larges ou minces, parfois dilatées aux bouts ; la pièce médiane variable ou manque 7
7 (18) Tergite anal noir luisant 8
8 (9) Les lobes membranaux sont triangulaires, longs et aïgus, au nombre de deux paires . . . . . **Pandelleisca** Rohdendorf
9 (8) Les lobes membranaux ne sont pas longs-triangulaires . . 10
10 (15) Les lobes membranaux sont larges ou en forme de crochets, souvent courts et rudimentaires 11
11 (14) La partie apicale du paraphallus a une pièce médiane et deux apophyses latérales 12
12 (13) La pièce médiane de la partie apicale du paraphallus est courte, large, en forme de bec et les apophyses latérales sont droites et bifides . . . . . **Jantiella** Rohdendorf
13 (12) La pièce médiane de la partie apicale du paraphallus est très développée, longue, large et de forme irrégulière ; les apophyses latérales sont arrondies aux bouts (fig. 1, A) . . . . . .**Occultophalla** gen. n.
14 (11) La partie apicale du paraphallus est dépourvue de la pièce médiane ; les apophyses latérales sont larges, courbées et pourvues d'une dent plus ou moins grande sur leur marge supérieure . . . . . .**Robineauella** Enderlein
15 (10) Les lobes membranaux sont bien développés 16
16 (17) Les styles sont lamelliformes, dirigés en avant et dépourvus de dents récurrentes (fig. 2, B) . . . . . .**Rosellea** Rohdendorf
17 (16) Les styles sont tubulaires, minces, longs, courbés en bas et pourvus d'un rang de dents fortes, longues et courbées (fig. 2, A) . . . . . **Varirosellea** Hsue
18 (7) Tergite anal rouge orange 19
19 (20) Les lobes membranaux sont rudimentaires, en forme de bosse. La partie apicale du paraphallus a les apophyses latérales fortement dilatées en forme d'une massue et dépourvue de la pièce médiane. Tergite génital rouge . . . . . **Jantia** Rohdendorf
20 (19) Les lobes membranaux sont bien développés 21
21 (22) La partie basale du paraphallus est fortement allongée. La partie apicale du paraphallus est grande, en forme de bouclier ; les apophyses latérales sont très minces et courtes. Les lobes membranaux sont longs, droits, fortement sclérifiés et courbés aux bouts. Tergite génital noir . . . . . **Curranea** Rohdendorf

22 (21) La partie basale du paraphallus est courte. La partie apicale du paraphallus a les apophyses latérales bien développées et une pièce médiane courte 23

23 (24) Les lobes membranaux sont très longs, dirigés distalement. La partie apicale du paraphallus a une pièce médiane indistinctement délimitée en angle. Tergite génital rouge . . . . . **Engelisca** Rohdendorf

24 (23) Les lobes membranaux sont larges et longs. La partie apicale du paraphallus a une pièce médiane délimitée. Le tergite génital a un tomentum dense sur sa partie postérieure . . . . . **Thomsonea** Rohdendorf

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SOME OBSERVATIONS OF RARE MATING CEREMONIES OF THE BLACK-WINGED STILT (*HIMANTOPUS HIMANTOPUS*) IN WETLAND OF CENTRAL SPAIN

J. P. GONZALES-KIRCHNER * and MARTA SAINZ DE LA MAZA

This paper describes some mating ceremonies of Black-winged stilt (*Himantopus himantopus*) observed during a long term in some humid zones of Ciudad Real (Spain), that concluded successfully and that presented remarkable modifications in comparison with the sequence described by other authors for this bird. The modifications observed refer in three cases to precopulatory movements and in one case to the postcopulatory movements. The mating ceremonies described in this note do not break the general model of ceremony described for the Black-winged stilt previously, but introduce little and punctual modifications that seem not to affect the success of the mating. Their low frequence of appearance can explain the absence of descriptions of this behaviour in other studies made with little number of observations.

INTRODUCTION

The Black-winged stilt (*Himantopus himantopus*) is a wader abundant in the South of Europe, where arrives from Africa to breed each spring. Its biology has been studied by different authors (3), (4). It presents, like the avocet (*Recurvirrostra avosetta*) a complex, and very showy, mating ceremony. In spite of that, this ceremony has been described only by 4 authors, and only based on a little number of observations; Benson (2) (one observation), Wilke (10) (one observation), Hamilton (9) (two observations) and Goriup (8) (six observations). These authors coincide in comparing the mating ceremony of the Black-winged stilt with the one of the avocet. They consider both ceremonies very similar and the significance in paircourtship probably the same (3). In this way, the mating ceremony of the Black-winged stilt follows the following sequence; "the female initiates the ceremony adopting the "soliciting posture", typically in shallow water, as the male passes. The male becomes very excited and starts to round the female passing behind her and stopping alternatively at each side of the female, where performs the "preening ritual" and/or "dip-shake display" at the level of the shoulder of the female. He repeats this cycle between 2 and 5 times before mounting the female by one side, flexing the legs and balancing by waving the wings allowing the copulation. After copulation the male dismounts and crosses his bill over the bill of the female and extends the wing over her back. Both consorts walk together for about 1 meter approximately, and after that, they separate, starting to preen themselves or to feed, without showing interest in one another" (3).

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METHODS

During the months of March to September of the years 1985, 1986 and 1987 we have carried out a study of the biology of the Black-winged stilt in the colonies of nidification placed in some humid zones of the province of Ciudad Real (Central Spain). Detailed descriptions of study sites can be found elsewhere (5), (6), (7). We have observed 177 mating ceremonies during the years 1985, 86 and 87. Table I offers the relative frequence of the mating ceremonies observed. We do not include here 2 trials made with inanimated objects, in one case with a stone and with a bottle in the other case. Special interest was devoted to those mating-ceremonies that present remarkable modifications in comparison with the sequences described by other authors.

RESULTS

Most of the mating-ceremonies observed by us take place in shallow water (Table I), in coincidence with Cramp (3), Goriup (8) proposed the association between success in copulation and the level of water where the mating ceremony take place. His observations of mating-ceremonyes of Black-winged stilt in Portugal indicate an association between shallow water and success in copulation. Our datta (Table I) seem to confirm this point. Table I offers the relative frequence of the mating ceremonies observed. 136 were successful, in the sense that finally the copulation was concluded, and 39 were unsuccessful because there was no copulation. A similar proportion of success/unsuccess was reported for the avocet by Andres (1) during a long term study made, about the sexual behaviour of this bird, in the Ebro delta (Spain). Most of the mating-ceremonies observed (97, 74%) do not break the general model of ceremony described previously. Into the mating-ceremonies that concluded successfully we have observed 4 that present remarkable modifications (Fig. 1) in comparison with the sequence described by other authors.

A) 6 of April 1985. Garbanzos Lagoon.

In a place with deep water, a female adopts soliciting posture when the male walks alongside her. The male responds rounding the female three times, passing behind her. At each side of the female the male makes dip-shake display at the sides of the female. On the second round, at the right side of the female, he preens his breast and after that he does all the third round on the right side of the female. In the both branches of this third round he makes dip-shake display and moves his legs on the soil. Mounts the female on the right side, balancing by the extension of his wings above and waving. After copulation they mantain their bills crossed and they walk together some steps before they separate.

B) 27 of March 1986. River Ojailen.

On wet soil the female adopts soliciting posture, and the male approximates rapidly from the water. The male moves around the female three times passing in front of the head of the female. At the end of

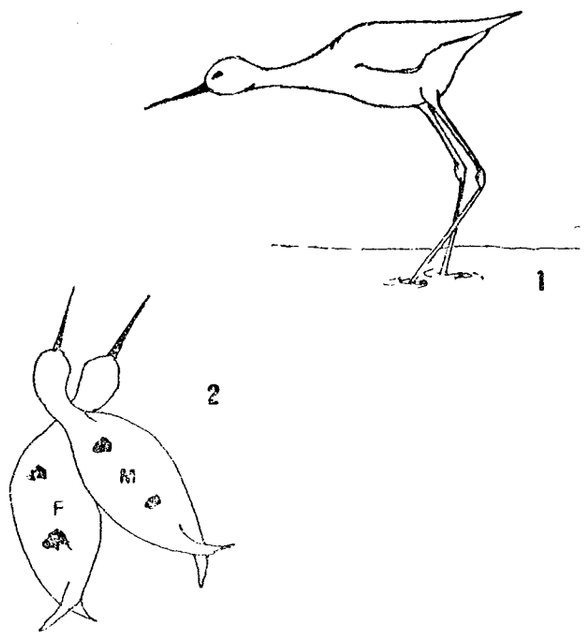

Fig. 1. — Postures and movements of *Himantopus himantopus* observed at Ciudad Real (Spain). Female (F), Male (M), Male movements around the female (dotted line); *1*, normal soliciting posture of the female; *2*, postcopulatory rare posture of crossed necks; *3* and *4*, precopulatory rare movements of the male.

each branch, at the side of the female, he makes dip-shake display and ritual preening. Mounts the female by the left side, without apparent problems of orientation. The copulation happens while the male balances opening its wings and extending them above and waving. After copulation both animals stand by, one to the side of the other and walk in this way, with their bills crossed, during some moments, and after that they separate.

C) 15 of April 1986. River Ojailen.

While the male feeds at 1.5 m of distance approx. of the female, in shallow water, the female adopts soliciting posture. The male comes near and does 4 semicircles around the female passing behind her. At the end of each round branch he does dip-shake display and preens his breast at both sides of the female. The male mounts the female by the left side balancing with its wings extended above and waving. After copulation they stay together crossing their necks. The female extends her neck to the male, and this one crosses his neck over the one of the female, touching the bill of the female at the left side of the female. After that they separate.

D) 30 of March 1987. River Ojailen.

The female adopts soliciting posture when the male walks near her in shallow water. The male moves around the female in a circle. After that he makes 3 semicircles passing in front of the head of the female, doing dip-shake display at the sides of the female with the head directed in opposite direction of the one presented by the female. The male mounts the female from the right side balancing with the wings partially extended in a 45° angle and waving. He touches the neck of the female with his bill. After copulation they stay together with crossed bills some instants before separating walking in a 90° angle.

DISCUSSION

The modifications observed affect in three cases, precopulatory movements (A, B, and D), and in one case, the postcopulatory movements (C) (Fig. 1).

In the case of the modifications of the precopulatory movements we can find two different types. One is represented by the case A. It has the typical sequence, except when the male does the last round, that was done completely at the right side of the female. It can be only a mistake due to the increasing excitement of the male. In fact, it was the last movement of the male before mounting the female.

On the other hand, the other two observations (B, D) seem to belong to a different phenomenon. The male modifies the trajectory of his precopulatory movements, that he does around the female passing in front of the female. Glutz et al. (4) speaking about the Black-winged

stilt, and Hamilton (9) speaking about the American avocet (*Recurvirostra americana*), say that the female moves in order to stay in the same direction as the male is moving, in the case that he tries to pass in front of her during the precopulatory ceremony. We have observed 9 cases of reorientation of the female like those described by Glutz et al. (4) and Hamilton (9). 7 of them ended in a successful mate, and also one case where there was no reorientation by the female and it ended without success when the male went away after passing in front of the female two times (La Perdiguera Lagoon, 8/4/86). Andres (1) found that a great source of failure in the copulations of avocet was due because of the male moves around the female incorrectly. All that seems to indicate that the movements of the male in front of the female are not an inexistent behaviour in the behavioural repertory of the Fam. *Recurvirrostridae*, although it appears in limited number of occasions and there is a strong component of repulse of the female against this behaviour of the male. The correction behaviour must take place on behalf of the female, that reorientates her body in the direction of movement of the male. Absence of this reorientative behaviour by the female, and incorrect precopulatory rounding movement of the male, combinated, produce these inverted mating ceremonies, in a very low frequence (Table I).

The only modification of postcopulatory movements observed also deserves attention. Cramp (3) describes variations in the postcopulatory movements of the male in the Black-winged stilt, which can do the ceremony of crossed bills, or dip-shake display before doing the postcopulatory race. Andres (1) found a 6,9 % of cases in the avocet courtship where there was no ceremony of crossed bills. Nevertheless, it has not been observed, in any of both species, the posture of crossed necks described here by us. It can probably be interpreted as an erroneous ceremony of crossed bills, but we do not have enough information to evaluate its significance correctly.

We can conclude that the rare mating ceremonies described in this paper do not break the general model of ceremony described for the Black-winged stilt previously, but introduce little and punctual modifications that seem not to affect the success of the mating. Two of them (B and D) are consequence of the combination of two rare behaviours; the incorrect direction taken by the male during the precopulatory movements around the female, and the absence of reorientation by the female as reaction to this behaviour of the male. Their low frequence (Table I) can explain the absence of descriptions of this behaviour in other studies made with little number of observations. The other two (A and C) correspond to irregular movements done into the typical mating-ceremony sequence shown by the Black-winged stilt. Studies made about courtship in avocets (1), (9), with a big number of observations, have registered also some irregularities.

Acknowledgements. We are very grateful to Dr. Vicente Ena that undertook a critical reading of the manuscript. Trixy Gonzales-Kirchner drew the illustrations from field notes.

Table 1

Observations of mating-ceremonies of *Himantopus himantopus* taking place each year during the study period, classified function of the substratum where they were done: A) Shallow water, B) Deep water, C) Wet soil. They are expressed in relative frequences of appearance (F), and number of observations (N). N and F values are expressed per year (1985, 1986 and 1987) and during the whole study (Total).

	Successful	Unsuccessful	Rare
	N : F	N : F	N : F
A	1985 : 38 : 0'76	4 : 0'08	0 : 0
	1986 : 53 : 0'71	7 : 0'09	1 : 0'013
	1987 : 36 : 0'67	5 : 0'09	1 : 0'018
	:		
	Total : 127 : 0'717	16 : 0'09	2 : 0'011
B	1985 : 0 : 0	2 : 0'04	1 : 0'02
	1986 : 2 : 0'02	3 : 0'04	0 : 0
	1987 : 1 : 0'018	2 : 0'03	0 : 0
	:		
	Total : 3 : 0'016	7 : 0'039	1 : 0'005
C	1985 : 0 : 0	5 : 0'1	0 : 0
	1986 : 0 : 0	7 : 0'09	1 : 0'013
	1987 : 3 : 0'05	5 : 0'09	0 : 0
	;		
	Total : 3 : 0'016	17 : 0'096	1 : 0'005

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OCCURRENCE OF *CORPORA ALLATA* IN *CLOËON DIPTERUM* (*INSECTA, EPHEMEROPTERA, BAËTOIDEA*) IMAGO, UNDER NORMAL AND EXPERIMENTAL CONDITIONS

A. SĂFTOIU

Corpora allata was described at larval stages of *Cloëon dipterum* from an anatomo-histological point of view. But its maintenance during quite long aerial mature stages, is still an open question.
We can signal not only that this endocrine gland normally persists the whole life-time, but also that a significant hypertrophy occurs in unfecundated, 18 — 20 days captive ♀♀. The investigations about the situation of this gland have been developed at both sexes imago in experimental conditions (captivity) or just collected from their natural medium. This fact actually proves the important physiological role of this species of Ephemeropterae.

L. Arvy and M. Gabe (5) signaled out an atrophy of *corpora allata* and of ventral glands with *Brachyptera risi* (Plecoptera) in the advanced larval stages and in imago. These data have to some extent imposed the idea that in insects with short imago life *corpora allata* is normally atrophying, having no physiological role any longer.

Referring to the gonadotrope function of *corpora allata* with insects, L. Jolly showed that allatectomy is indifferent to both young oocytes in previtelogenesis and to the advanced eggs, while the oocytes at the beginning of vitelogenesis are sensitive to allatectomy, degenerating consequently to such an ablation.

All these data reinforced the supposition that, generally with Ephemeropterae during their aerial stages, *corpora allata* would no longer have any role to play and most likely it is expected to get atrophied with the very last aquatic, nimphal stage, when oogenesis is already over.

Later on, M. Gabe (12), investigating the neurosecreting processes with the Ephemeropterae, suggested that a possible study subject could be still the situation of the endocrine glands with imago ephemerae.

It is also known, than out of Giard's studies, the poecylogonia with *Cloëon dipterum* that behaves like an oviparous species in Northern areas and like viviparous species in Southern ones; in the second case, the females retreat after eclosion and swarm to reclusive places, for eggs incubation. The entire period of embryo development lasts for 3 weeks.

Considering these data, we have made several preliminary observations evidencing that if as for the ventral glands, involution up to total resorption is often met with Ephemeropterae (*Palingenia longicauda*), for *corpora allata*, there is a different development both in *Cloëon dipterum* and in other ephemerae.

MATERIAL AND METHODS

In order to get the experimental groups consisting of imago mean to be kept into cages under laboratory conditions, initially *Cloëon dipte-*

rum advanced nimphae were collected, out of which subimago eclosed These were removed to entomological thinscreened, in high cages; the cages were placed into shady rooms at constant temperature during the entire experiment and at normal humidity.

Advanced nymphs were collected at the end of summer, in September, thus obtaining a material more resistent to experimental conditions, namely to show low rate of mortality to eventually 0.

Mainly ♀♀ of *Cloëon dipterum* were kept into the cages, unfecundated and in absence of ♂♂. The latter resist even less to experimental conditions, showing a higher mortality.

Females constituted the main subjet of the present paper also because of the hypothesis according to which a functional correlation between *corpora allata* and the events occurring in the gonads would operate during this terminal stage.

In order to complete the biological material and, at the same time, to enable the comparison of results, imago have bean collected from nature, in different physiological states: ♂ imago and recently eclosed ♀ ones, during swarmings of acculplation, ♀♀ with embryos at the beginning of their development within the incubating chambers or ♀♀ depositing their larvae in water, being thus at the final period of imaginal life.

The captive females in the enthomological cages did not manifest any kind of activity, usually resting on the screen walls, seldom changing their places by nocturnal movements. Out of them fixations were made sampled at intervals of: 3, 5, 7, 9, 13, 15, 17, 19 — 20 days, the maximum lenght of imaginal life being of at most 21 days. Fixations were made in Bouin (with neuter formol), and the histological sectionings, after having been paraffin included, were stained with Haematoxiline-erythrosine, Alcian-blue, or with Paraldehyde-fuxine, in order to allow the parallel study of the development of neurosecretory phenomena, particularly the protocerebral ones and storages of neurosecretory product in *corpora cardiaca* as well.

RESULTS

Corpora allata with *Cloëon dipterum* is a compact organ, without lumen, made up of 3 — 6 lobes with a relative bilateral symmetry, occupying the space between the suboesophagian ganglion and the anterior intestine (fig. 1). Allatal lobes are free only at their extremities (apical and caudal) confluencing at the median central region constituting the most massive part of the gland, dorsally joining the suboesophagian ganglion.

Free lateral areas from the lobes of *corpora allata* may raise along the edges of the anterior intestine only for a short route (the first ventro-lateral third).

The inner, cephalo-thoracic pressures caused by ovaries egg filled or larvulae in development (with ♀) or intestine with air with aerostatic role (with imago of both sexes), force *corpora allata* to insinuate itself in the spaces between the kitinous cephalo-thoracical crestes and tracheean, main trunks, descending from the trachean node constituted by the Palmen organ, and thus showing a variable anatomical topography.

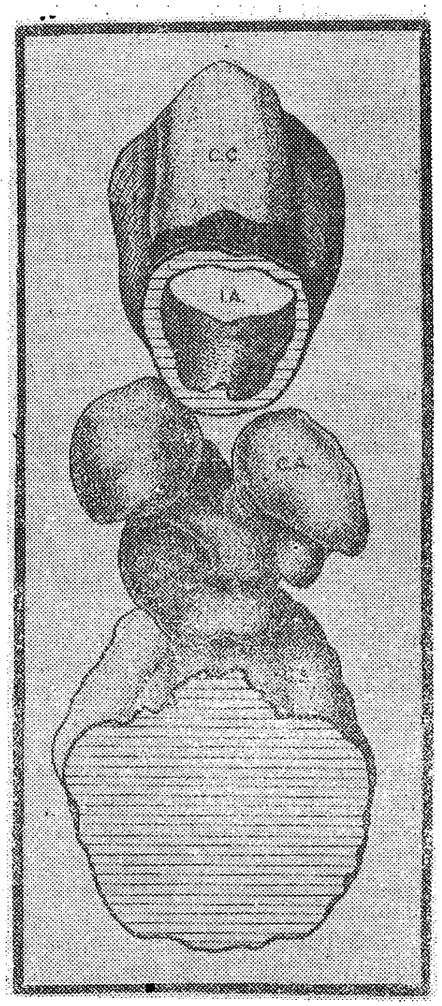

Fig. 1. — Anatomical placement of *corpora allata* in imago of *Cloëon dipterum*: G.S. — —subóesophagus ganglion; I.A. — front intestine; C. C. — corpora cardiaca.

On an early stage (aquatic larvo-nimph), the anterior allatal lobes are free for a rather large distance, then join into a single median body that is caudally continued. The thickness of the lobes is about 30 μ, and the antero-posterior length, of 112 μ. The thickest areas are made up of 4 — 5 cell layers and the thinnest of 2—3. Cellular cytoplasm is 1—4 μ with perinuclear width, and nuclei are of 3 μ Ø. On the first aerial stage (♂ subimago) the two lobes have their ends thickened, made up of 5 — 6 cell layers each, disposed in cordons without any precise orientation. Nuclear diametera reach 4 — 5 μ, and the cellular cytoplasm is now denser (8 μ).

On ♂ imago stage, whose aerial life is most often short, its role in accuplation being achieved immediately after eclosion, *corpora allata*

occupy more completely the space from below the anterior intestine, the allatal lobes being tightly stuck to the dorsal surface of the ganglion. The central lobe is much enlarged, and in the allatal cells appear thin vacuolizations, the 3,5 μ nucleus presents a clearer nucleol and less chromatic stuff, both difused or reticulated.

But on the ♀ aerial stages, the developments of *corpora allata* are more specific. On recent imago with their abdomen filled with mature unfecundated ovules, *corpora allata* starts from the anterior part through three lobes (two lateral more dorsally positioned and a median one supraganglionar positioned) that after a short route, confluence into a central mass, which in its turn has got several free lobular protuberance. This central mass ends up caudally, into a sole lobular protuberance (fig. 1).

Compared to the ♂ imago, *corpora allata* on ♀ is visibly bigger even now, the most enlarged sections having the very same surface as the suboesophagian ganglion (120 μ). Nucleii of allatal cells are 5 — 6 μ Ø, the fuxinophile nuclear mass centralizing itself in time while the remaining of the chromatic material is disseminated into small, spherical, peripherically disposed islands. The dorso-ventral thickness of the gland is about 100 μ, and the more developed lateral lobules reach a bigger height on the ventrolateral sides of the intestine.

From the neurosecretory point of view, with these recently eclosed ♀♀, the median neurosecretory cells, from *pars intercerebralis* are filled up with the product, that, at this point cannot be found stored in *corpora cardiaca*. Two addional pairs of n.s.c. in the mezothoracic ganglion are also active. On this neurosecretory background the developments of *corpora allata* do take place.

Up to several days (5 — 7) of age, in virgin ♀♀, kept in captivity without ♂♂ there were no significant modification with *corpora allata* (fig. 2, 3). The cellular limits are clear, distinctly showing a disposition into semiorder cordons with a vague parallel stratification as related to the dorsal face of the suboesophagian ganglion. Many allatal cells are alongated, the nucleus being of 4—5 μ in Ø, and nucleol reaches a 2—2,5 μ diameter.

With 10-days ♀ imago, *corpora allata* has an antero-posterior extension of about 110 μ, the 5 — 6 component lobules getting closer. Only small portions remain separate and free, the rest getting compact by confluence or simply through a very tight closeness (fig. 4). The biggest allatal cells get to a 10 μ Ø, with a dense and uniformly granulated

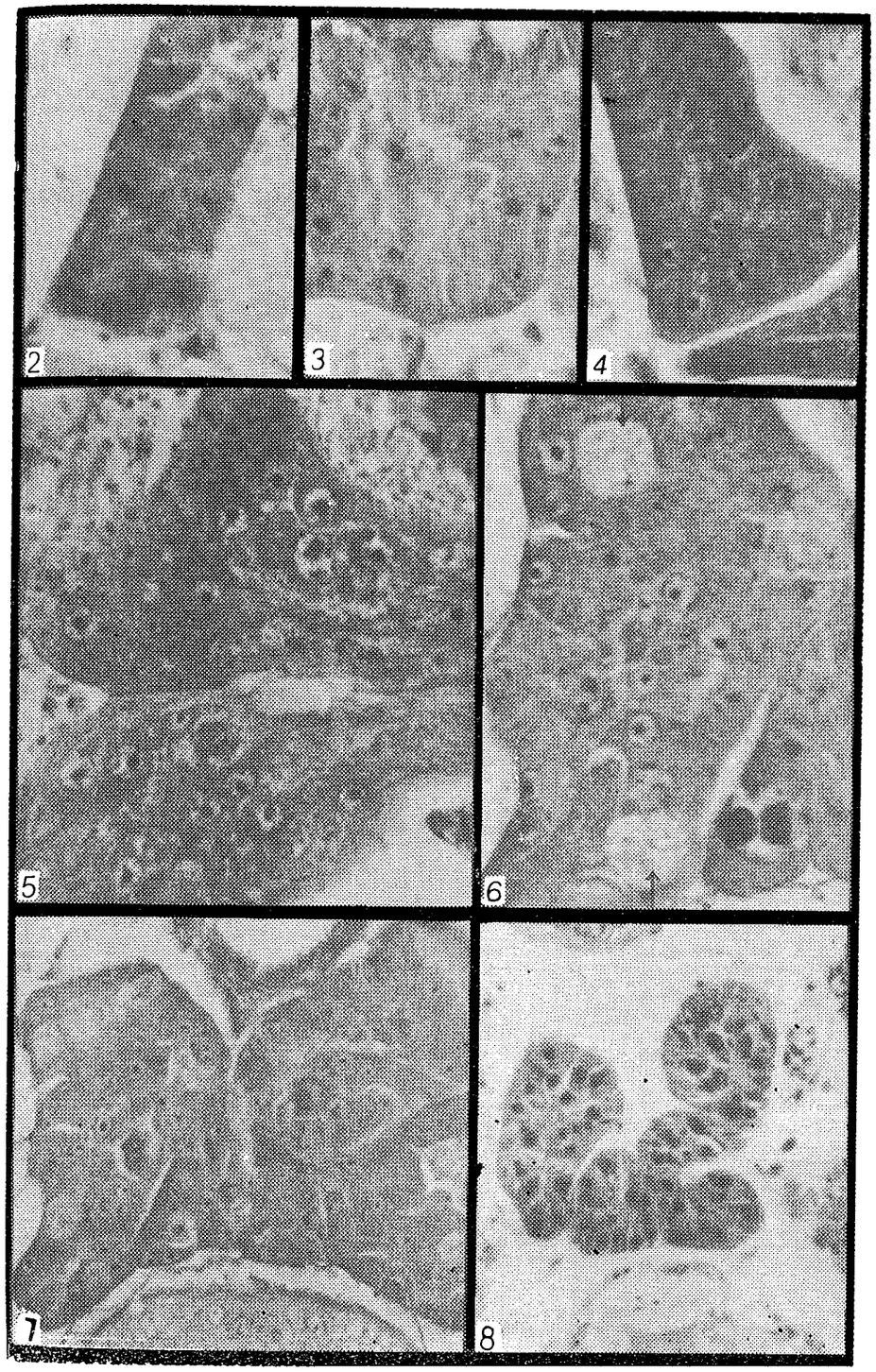

Fig. 2—8

Fig. 2. — 8 — Histological structure of corpora allata in ♀♀ imago kept under experimental conditions. Fig. 2 — section through the allatal lobe in 5 day-imago, Bouin, Bleu-Alcian, 400 x; fig. 3 — in 7 day-imago, B. Bl.-Alc., 400 x; fig. 4—in 10 day-imago, B. Bl.-Alc 400 x; fig. 5—in 13 day imago, B. Bl.—Alc. 400 x; fig. 6 — in 18 day-imago, B. Bl.-Alc. 400 x; fig. 7 — in 20 day-imago, B. Bl.-Alc. 400 x; fig. 8 — in recently imago collected from natural medium, Dubosque-Brazil, Hematox.-erithrosine, 200 x.

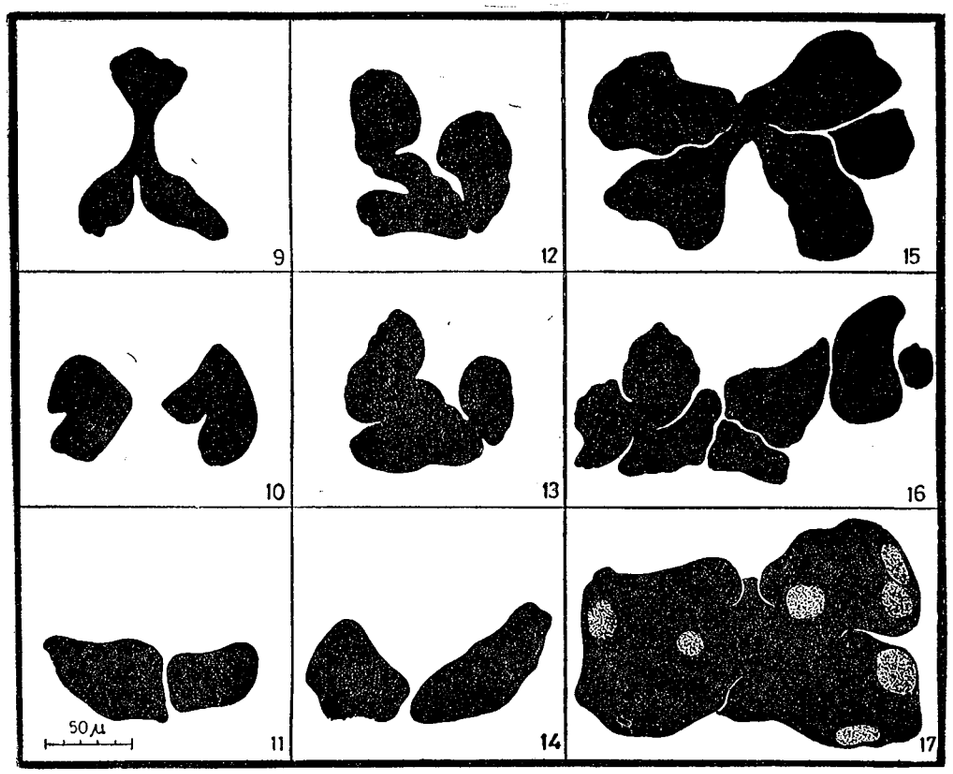

Fig. 9 — 17 — Maximum sections through *corpora allata*, with *Cloëon dipterum* at different physiological stages and moments: fig. 9 — ♂ larvonymph; fig. 10 — ♂ subimago; fig. 11 — ♂ imago; fig. 12 — recently ♀ imago; fig. 13 — advanced ♀ imago; fig. 14 — ♀ imago depositing their larvae in the water. Fig. 15, 16, 17 — experimental group :♀ imago recently, unfecundated (fig. 15), 10 day ♀ imago (fig. 16), 20 day ♀ imago (fig. 17). It is worth noticing the significant hypertrophy of *corpora allata*.

cytoplasm, without any vacuolizations or free areas. The very uniform nuclei reach a max. 5 µ in Ø and have big nucleols also placed centrally.

With 13-days ♀ imago of captivity (fig. 5) hypertrophy of *corpora allata* starts. The total antero-posterior lenght is of 190 — 200 µ, the nuclei slightly exceeding 5 µ in Ø, and the fuxinophyly of the nucleolus gets more evident the cytoplasm maintaining its fine granular and uniform appearance.

Towards the end of imaginal life in captivity, with 20-day ♀♀ this hypertrophy of the gland appears even more evident (fig. 7). Sectionings through the central areas get to the width of 240 µ, height of 120 µ, thus exceeding, by far, the diameter of the suboesophagian ganglion diameter (120 µ). The antero-posterior length is now of 165 — 200 µ. Within the surface of a maximum sectioning, 50 — 60 nuclei can be counted that can reach 6 µ diameters, the nucleole still centrally situated. These cells that represent the highest percentage, have their cytoplasm unchanged as regards its aspect. But some 12 — 15 cells appear modified, as if passing through the several phases of a vacuumization process finally leading to complete emptying (fig. 6). During the first phase, cytoplasm in these cells is less compact, partially losing their tinctorial affinities. This cytoplasm is now a finely reticulated trama with a slight fuxinophyle inclination, the nucleus remaining with a normal aspect (fig. 6). Several cellular spaces appear totally emptied, and, within the perimeter of the other, just some deposits consisting of spherical refringent and fuxinophyle granules piles, occupying an oval 5—6µ Ø areas are left behind. Within the space of an allatal cell another type of dense deposit appears but seldom geometrically shaped, a cytoplasmatic contraction like by its homogenous structure and its affinities, but which may be rather a residual body. The allatal vidated cells have generally a diameter rancing between 12 — 15 µ, being thus very big cells.

This hypertrophying process of *corpora allata* accompanied by the above described cellular modifications, doubtlessly indicate signs of experimentally induced functional alterations, since this gland cannot normally pass through such a long period of inactivity, waiting for the accoupling moment and beginning of some metabolical process requiring the potential endocrine adjustment.

Investigating, for comparative purposes, the *corpora allata* with ♀♀ imago of the species captured from nature, but bearing in their incubating chambers fully developed embryos, and a characteristic pigmentation, thus apparently being done towards the end of the 20 — 21 the day of imaginal life, it can be found that this endocrine gland does not reach exaggerated sizes, keeping itself close to the initial sizes (in the first days of imaginal life). Allatal cells, without showing picnotical phenomena, present normal appearances, a uniform cytoplasm, the nuclear dimensions being only slightly decreased. No cellular vidations or residual bodies are observed (fig. 8), as with the experimental cases. As the imaginal life is coming to an end, it can be concluded that this endocrine gland (corpora allata) *is present and functional during the whole period of the imaginal life.*

DISCUSSIONS

Referring to the chosen experimental situation, several aspects of the study undertaken by P. Allegret (2) on the functioning of *Galleria melonella* (Lepidoptera, Pyralidae) should be mentioned here. The adult of this species has a short imaginal life, during which it does not feed itself. The ovarian evolution had taken place during the nimphal stage, thus oogenesis was over at the imaginal eclosion and the mature oocytes were able of being fecundated. This situation is similar to the in ♀ of *Cloëon dipterum*.

P. Allegret's experiment keeps isolated ♀♀ virgins of Galleria to which it also stops the eggs lying, obturing the oviopositor. This experimental retention had as a global consequence an advancing females longevity to the maximum value, leading to the stagnation of several processes in the ovary morder in wait of some events to enable the natural functioning. Mechanisms of ovocytary destruction, that with many insects but with a longer imaginal life are both cyclical and under hormonal control, are thus hindered to express themselves — Jolly described this aspect with Dytiscidae (16).

With *Galleria mellonella* and with Ephemeropterae, due to the relatively short imaginal life, there is only one reproductory cycle, the one prepared during nimphal life, that now with imago lead to the stage of mature oocyte filled ovaries, capable of fecundation. Artificial discontinuity of expressing this end of reproductory cycle can't bring about anything else but physiological mechanisms leading only, on a first stage, to an increased longevity, since another reproductory cycle is out of question.

With genuine Ephemeropterae, with short imaginal life (a few hours only), the ovarian cycle wholly completed from the aquatic larvonimph stage, in the very moment of eclosion, is accompanied by all neuroendocrine phenomena and it is rapidly coming to its end, after nuptial flight and accoupling, by deposit of eggs. It is difficult to suppose that with these species there might still exists any stopping or postproning posibilities as regards all these processes as with *Cloëon dipterum*. Practically, the possible oocytary stagnation athresia or involution processes and ovarian resorption do not have the necessary time to occur any longer, within a much too short imaginal life.

Thus the extreme survival of Cloëon ♀♀ kept in captivity, without ♂♂, may be on some neuroendocrine adjustments where an important role could be played by the protocerebral neurosecretion and *corpora allata*. During the whole imaginal life of these ♀♀, *corpora allata* shows the histological signs of a gradual increase, distinctly expressing itself in the 20th day. These developments involve *c. allata* in maintaining a certain state of the gonads during the whole period and can even explain the Neopter type adaptation of an imaginal life, or of larvules incubation and viviparity. The normal state of *Cloëon dipterum* females, fecundated since subimaginal eclosion, that also cross a great many days, involve, as proved by the histological signs, *corpora allata* itself. But the imposed experimental conditions, brought about the evidence of some specific aspects related to the development of this endocrine gland (Figs. 9—17).

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REGULATION OF THE CIRCADIAN RHYTHM OF FOOD INTAKE AND OF THE ENERGY METABOLISM OF RATS

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Repeated light-dark cycle changes in rats do not affect the lenght of the latent period for re-intrainement of the eating rhythm. The latent period is shortened only after continuous light or darkness. Continuous darkness does not affect the eating rhythm, which is gradually abolished by continuous illumination within 10 days.
Energy metabolism presents a rhythmic activity, having 2 peaks and 2 minimum. This rhythm is maintained in rats even after 24 hours of starvation.
Isolation of the medial hypothalamus does not affect energy metabolism rhythm.
It is concluded that different physiological rhythms functions are controlled through different ways.

As a night animal, the rat consumes in the light period about 20 — 30% of the whole amount of its food, the rest being consumed at dark period (3). The present experiment aimed to establish : the role played by light, considered as a basic external synchroniser of endogeneous mechanisms controlling biorhythmicity ; the transition period for re-intrainement of the eating rhtyhms and energy metabolism, as well : the influence of conditioning upon the latent period. Having all these in view, the food intake and energy metabolism have been established after repeated changes of the light-dark rhythm, or after continuous dark or illumination, establishing each time the duration of the latent period.

In parallel, the role played by the medial hypothalamic afferences upon the control of the functions' rhythm, has been established.

MATERIAL AND METHODS

The experiments have been performed on male rats, weighing about 230—250 g at the beginning of the experiment. They have been fed according to the McCollum diet and water ad libitum. The rats have been kept in metabolic cages of the Rufeger-type, while the food intake has been measured at 7 a.m. and 7 p.m., respectively. The regime of illumination is indicated in figures. The energy metabolism has been determined by indirect calorimetry, the respiratory exchanges being measured interferometrically.

Isolation of the medial hypothalamus has been performed stereotaxically, under nembutal anestesia, by using a knifle having the form of an inverted cone, with a radius at the base of 2 mm, while the horizontal part has been placed 2 mm upward. The isolation of the median hypothalamus began just behind the optic chiasma, and posteriorly it included part of the mammillary bodies. The results were analysed statistically, using Student's t-test.

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RESULTS

1. *Influence of the repeated changes of the light-dark rhythm upon the latent period.* Shifting the dark 7AM—7PM illumination regime to the light 7AM—7PM one induces inversion of the food intake rhythm after 6 days (fig. 1.). The length of the latent period remains the same after shifting from the light 7AM—7PM illumination regime to the dark 7AM —7PM one. Repeated light-dark cycle changes do not affect the length of the latent period for re-entrainment of the eating rhythm (fig. 1.).

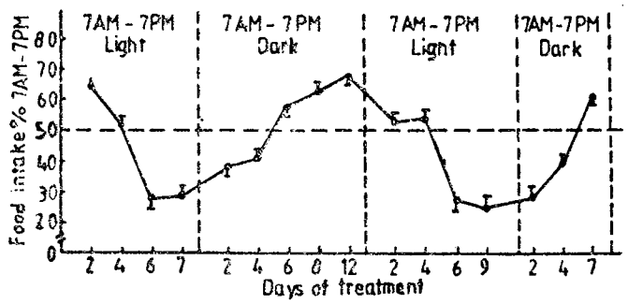

Fig. 1. — The effect of repeated light-dark cycle changes on the length of the latent period for re-entrainment of the eating rhythm in the rat. Values are M± SE. Number of rats = 12.

2. *Influence of continuous illumination upon the food intake rhythm.* The continuous illumination leads to the disappearance of the eating circadian rhythm in about 10 days. Shifting the animals from continuous illumination to a 7AM—7PM light illumination regime, 7AM—7PM dark or vice-versa, induces a recovery of the eating rhythm after a latency of only 4 days (fig. 2).

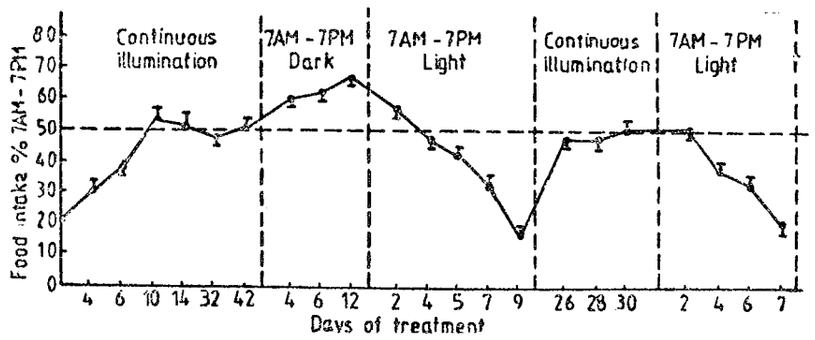

Fig. 2. — The effect of continuous light on food intake rhythm.

3. *The influence of continuous dark upon food's intake rhythm.* Moving of the rats from daytime dark to continuous darkness maintains the same consumption rhythm (fig. 3). Reintroduction of rhythmic illumination causes reappearance of the rhythm of food intake in 4 days.

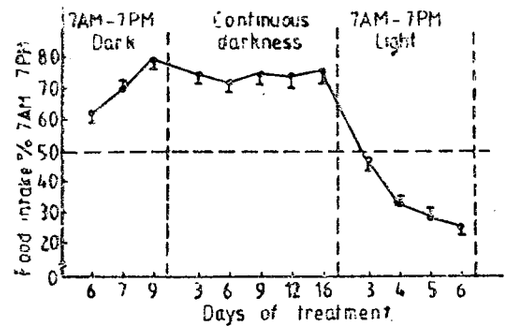

Fig. 3: The effect of continuous darkness on food intake rhythm.

4. *The influence of reverted illumination rhythm on the energy metabolism.* Normal rats, kept at 7AM—7PM light, evidence 2 peaks of the rhythm of their energy metabolism, as follows : one having a lower value, placed at 12 o'clock and the other, with higher value, placed at between 20 and 24 pm, as well as two minimum values : at 16 and 4—8 o'clock respectively (fig. 4). After inversion of the illumination regime, change of the rhythm of the energy metabolism is recorded after 8 days, a maximum being recorded at 16 and a minimum al 12 o'clock (fig. 4).

5. *Influence of inanition.* Starvation of rats did not affect significantly the rhythm of the energy metabolism, a minimum being recorded at 16, and a maximum at 20 o'clock (fig. 4).

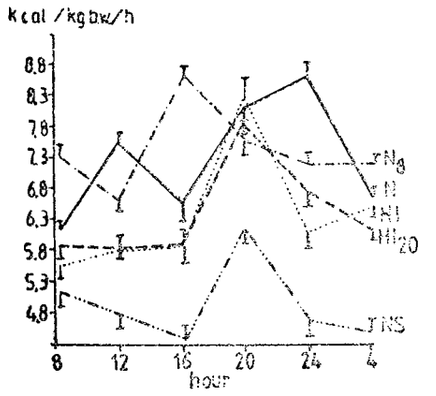

Fig. 4. — Circadian rhythm of energy metabolism in normal (N), starvated (S) or medial hypothalamus isolated (HI) rats maintained under light from 7AM-7PM. The pattern of food intake rhythm after 8 days or 20 days reverted lightning conditions (light : 7PM-7AM) in normal (N_8) or with hypothalamic isolation (HI_{20}) rats. Number of rats = 6.

6. *Influence of isolation of the medial hypothalamus.* The maximum recorded at 20 is maintained even after the isolation of the medial hypothalamus. When the illumination rhythm is changed, the rhythm of the energy metabolism of the operated animals is not modified, even after 20 days (fig. 4).

DISCUSSION

The results obtained evidence that, in the case of rats, the lenght of the latent period for inversing the biorhythm of the food intake is not modified by training. It decreases only when shifting from a rhythmical illumination regime subsequent to continuous illumination or dark. Also, the disappearance of the rhythm of food intake is to be observed only after continuous illumination, which is not the case with continuous dark.

In the case of humans and animals with a higher developed nervous system, it is considered that, besides the inborn mechanism of temporary synchronization of the circadian rhythms type, a rhythmical stereotype of functions is also elaborated during their lifetime, superposed over the inborn rhythms (1), (2). Persons specially trained for the frequent modifications of the work-rest regime are better adapted, as compared with untrained ones, which permits the conclusion that the structures involved in the elaboration of the time conditioned reflexes are related to the inborn circadian "clocks". Our experiments have demonstrated that the repeated inversion of the illumination period does not affect significantly the duration of the transition period of inversing the food intake's rhythm. Possibly,this might be due to a relatively constant time required for the "substance(s)" synthesis,known as cotrolling the rhythmicity of certain functions.

A main role in the determinism of biorhythmicity is attributed to the suprachiasmatic nuclei (SCN), that functions as an endogeneous synchronized mainly to the day-night cycle, and regulating the rhythmicity of the melatonine synthesis to the signals given by the light's circadian duration. Thus, measurement of the photoperiod time by the circadian biorhythm of melatonine becomes possible. Light inhibits the synthesis of melatonine on a nervous path. The optical signal from the retina reach SCN through the retino-hypothalamic and geniculato-hypothalamic tracts; from here, the SCN fibers pass through the paraventricular nuclei (PVH) and the tuberal hypothalamus into the lateral hypothalamus (4), which has synaptic connections with the preganglionary neurons from the superior thoracal segments. The preganglionary fibers synapsize into the superior cervical ganglion, while the postganglionary ones reach the pineal gland through the conarii nerves, but also through several fibers that innervate initially the habenula.

Apart from innervation of the pineal gland, described above, there also exist cerebro-pineal fibers having their origin in PVH, as well as in the nuclei of the habenula, those of the posterior comisure and in the lateral geniculate bodies. Through PVH there pass the two pinealopetic paths, that is, both sympathetic medular and commisural path, through the direct hypothalamo-pineal fibers. Noradrenaline, released at the postganglionary fibers endings, stimulates the secretion of melatonine. Light inhibits the discharge ratio of noradrenaline from the postganglionary fibers in the pineal gland. The inversion time of circadian rhythm of serotonine secretion—finally converted into melatonine is of about 7 days (5). As this time is relatively constant, it might explain the constancy of the latent period of inversing the food intake rhythm, as a consequence of the repeated modification of the illumination period.

The shorter duration of the transition period (4 days), following a constant illumination regime, is probably determined by the appearance of certain modifications at level of the pineal gland, during constant illumination, modifications to be completed after the installation of a rhythmical regime of illumination. In the case of rats, maintainance of the food intake rhythm in continuous dark, might be possibly determined by the maintainance of the melatonine secretion rhythm through endogeneous mechanisms, as dark does not inhibit the secretion of melatonine.

The energy metabolism of rats has a rhythmical activity, the peaks being recorded in the dark period, rats being nocturnal animals. The relatively high values recorded at 12 o'clock might be due to a partial adaptation of rats to day light.

The circadian rhythm of the energy metabolism is not caused by the rythm of the food intake, as the evening maximum is recorded in rats even in the absence of digestive activity.

Inversion of the illumination rhythm modifies the rhythm of the energy metabolism after about 8 days, which represents a latent period close to that of food intake rhythm or of serotonine rhythm.

Isolation of the medial hypothalamus induces a sudden abolition of the food intake rhythm (3), which may be probably due to sectioning of the fibers passing through PVH, while the same hypothalamic intervention does not affect the rhythm of the energy metabolism. Consequently, the conclusion may be drawn that, on one side, the rhythm of different functions is controlled by different mechanisms and, on the other side, the rhythm of the hypothalamic neurosecretions (2) does not affect the rhythm of the energy metabolism although, probably, it is necessary for the synchronisation of the rhythm to the modified conditions of action of the external synchronizer — in our case, the light. As a consequence of the isolation of the medial hypothalamus, known as abolishing the rhythm of neurosecretions (2), the rhythm of energy metabolism cannot be modified, even after 20 days from the reversion of illumination.

As a general conclusion, it seems that the relatively constant duration of the latent phase, for the re-instalation of a rhythm depending on the modification of the illumination rhythm, might be induced by the time required to reverse the melatonine synthesis rhythm, known as further acting, by means of the melatonine receptor from SCN, basic in the regulation of biorhythmicity.

From a physiological point of view, it is our opinion that the resynchronisation of functions, for workers used to work in shifts, is not accelerated, which might impose a longer duration of one shift, permitting the synchronisation of the rhythm of the organism's various functions.

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REPRODUCTIBILITY OF THE ANTITUMORAL ACTION OF THE PA_2 III AND PA_3 POLYPHENOLIC PREPARATIONS

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Three successive tests were performed, in identical experimental conditions with those that leaded to the antitumoral activity evidence of the PA_2 III and PA_3 polyphenolic preparations, on rats bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma. The values of the evaluation indices were analyzed comparatively with those standard, imposed by the reference programs for this step of the preclinical screening. Thus, we revealed the reproducible and stable character of the cancerostatic pharmacotherapeutical effect of the studied products. The qualitative evaluation of the antineoplastic action requires its quantitative evaluation by numerous and interdependent investigations. These will assure or not the estimation of the antitumoral therapeutical effectiveness significance of the PA_2 III and PA_3 polyphenolic preparations.

The preclinical screening chemotherapeutic programs aiming to identify new cancerostatic agents, require a complex and multistage investigation of the action of some drugs with supposed antitumoral effects on malignant development process, which should assure the qualitative and quantitative evaluation of specific pharmacological action on adequate experimental models (1—6), (8—10), (12—14).

In a previous work we reported the antitumoral pharmacotherapeutic effect of the PA_2 III and PA_3 polyphenolic preparations on Guérin T—8 lymphotropic epithelioma and Walker 256 carcinosarcoma (7).

In the present paper are exposed the experimental results obtained in the successive testing circuit of the PA_2 III and PA_3 antitumoral action. They are necessary to appreciate the reproducibility and stability of the specific pharmacodynamics effect.

MATERIALS AND METHODS

Three successive tests were performed in the same experimental conditions as in the preliminary investigations, which highlighted the antitumoral pharmacotherapeutic effect of the PA_2 III and PA_3 polyphenolic preparations. These conditions are : same experimental tumoral systems ; same therapeutic dose ; same program and pattern of antitumoral treatment.

White Wistar female rats of 125—150 g bearing either Guérin T—8 lymphotropic epithelioma or Walker 256 carcinosarcoma were used as experimental animals, both tumor lines being of solid type.

The cancerostatic treatment, started 24 hours after the tumoral transplant, was applied daily by intraperitoneal (i.p.) injection of the PA_2 III and PA_3 polyphenolic preparations in a dose of 5 mg/Kg. body

weight (b.w.) and of 45 mg/kg b.w., respectively. This therapy lasted for 16 days in the case of Guérin tumor and 19 days in the case of Walker tumor.

The estimation of the antitumor effect was based on the comparative follow up of the mean tumor weight (MTW) in treated and control animals at sacrifice.

The evaluation of antineoplastic action was made by the percentage determination of mean tumor regression (% MTR) and by the calculation of the T/C value (where T = MTW for the treated groups and C = MTW for the control groups) (5), as well as of the statistic significance using the Student's "t" (11).

The demonstration of the pharmacotherapeutic effect reproducibility has involved the assessment of some specific indices, too (5):

— the T/C × 100 value of the retests;

— the superior and inferior limits of the admissible variability range, established on the basis of the formulas T/C × 100 × 1.82 and T/C × 100 : 1.82, respectively (the T/C × 100 value represents the one of the first test);

— the T/C values products of the first two tests;

— the T/C values product of all tests.

The appreciation of the antitumoral pharmacotherapeutic effect reproducibility was performed by the comparative analysis of our evaluation indices and of those imposed by the selection criteria of active cancerostatic substances. These criteria were established by the preclinical screening programs of the Cancer Chemotherapy National Institute from U.S.A. (5) and of the Institute for Microbiology and Experimental Therapy from Germany (3) for this stage of the antitumoral action qualitative evaluation.

RESULTS

The successive testing of the PA_2 III polyphenolic preparate action on the development of Guérin T—8 lymphotropic epithelioma has conditioned the results included in table 1.

Table 1

Successive testing of the antitumor activity of the PA_2 III polyphenolic preparation therapy (5mg/kg.b.w./i.p./daily) on rats bearing Guérin T-8 lymphotropic epithelioma. Figures in brackets indicate the number of animals.

Group/ Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	12.4±1.8(15)	—	—	—
PA_2 III	6.1±1,6(10)	50.8	0.49	p<0.01
CONTROL	12.8±1.4(14)	—	—	—
PA_2 III	5.8±1.1(10)	54.7	0.45	p<0.002
CONTROL	15.8±1.6(15)	—	—	—
PA_2 III	8.0±1.2(10)	49.4	0.51	p<0.002

In the initial experiment, it can be observed that the antitumoral therapy with PA_2 III has induced a significant decrease of MTW ($p < 0.01$) in comparison with the control group. This cancerostatic action is expressed both by MTR percentage value (50.8%) and by T/C value (0.49).

The T/C × 100 value of the initial test — necessary to establish the admissible variation range — was of 49%. Its upper and lower limits are of 89.2% and 26.9%, respectively.

Also, as compared to the controls, it can be seen that the first retest was correlated with a MTR of 54.7% and a T/C ratio of 0.45. The values of the evaluation indices confirm the significant antitumoral potential of the PA_2 III. The T/C × 100 value of this retest is 45%.

The T/C values in the two tests allow an estimation of their product of 0.22.

Finnaly, the second retesting was also materialized by an antitumoral effect of PA_2 III preparation, the evaluation indices being: MTR of 49.4%, T/C value of 0.51 and T/C × 100 value of 51%.

The product of the T/C values in the three successive tests is 0.11.

The results obtained with PA_2 III polyphenolic preparate, in the context of the same experimental protocol, on rats bearing Walker 256 carcinosarcoma, are presented in table 2.

Table 2

Testing and retesting of the cancerostatic effect of the PA_2 III polyphenolic preparation treatment (5mg/kg.b.w./i.p./daily) on rats bearing Walker 256 carcinosarcoma. Figures in brackets indicate the number of animals

Group/ Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	12.2±1.5(14)	—	—	—
PA_2 III	6.1±10.(10)	50.0	0.50	p<0.01
CONTROL	14.6±1.3(14)	—	—	—
PA_2 III	7.7±1.5(10)	47.3	0.53	p<0.01
CONTROL	15.2±1.0(15)	—	—	—
PA_2 III	7.8±1.4(10)	48.7	0.51	p<0.001

It can be ascertained, as compared to control group, that the rats submitted to the daily treatment with PA_2 III presented at sacrifice a significant decrease MTW, which allows the assessment of the following values of the induced antitumoral action:

— MTR of 50% and T/C ratio of 0.50, for the initial experiment;

— MTR of 47.3% and T/C ratio of 0.54, for the first retesting and

— MTR of 48.7% and T/C ratio of 0.51, for the second retesting.

The corresponding T/C × 100 values are: 50.0%, 53.0% and 51.0%, respectively.

The limits of the admissible variation range, calculated on the basis of the initial T/C × 100 value, are: the maximum one of 91.0% and the minimum one of 27.5%.

The product of the T/C values of the first two tests is 0.26 and that of the all three tests is 0.13.

The reproductibility of the antitumoral pharmacotherapeutic effect of the PA_3 polyphenolic preparate was followed by successive tests performed on both experimental tumoral systems.

Table 3 presents the results obtained when the antitumoral action of the PA_3 was tested and retested on rats bearing Guérin T—8 lymphotropic epithelioma.

Table 3

Successive testing of the antitumor activity of the PA_3 polyphenolic preparation therapy (45mg/kg.b.w./i.p./daily) on rats bearing Guérin T-8 lymphotropic epithelioma. Figures in brackets indicate the number of animals

Group/ Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	15.8±1.6(15)	—	—	—
PA_3	6.6±1.2(10)	58.2	0.42	$p<0.001$
CONTROL	12.8±1.4(15)	—	—	—
PA_3	5.9±1.1(10)	53.9	0.46	$p<0.001$
CONTROL	12.4±1.6(15)	—	—	—
PA_3	5.5±1.0(10)	55.7	0.44	$p<0.001$

In comparison with the control groups, it is observed that this treatment has induced a significant decrease ($p < 0.001$) of MTW, which allows an estimate of MTR values of 58.2% 53.9% and 55.7%, respectively. The corresponding T/C values are 0.54, 0.55 and 0.54, respectively. The T/C × 100 values are: 42%, 46% and 44%.

Knowing that the T/C × 100 value of the first test is 42%, the calculated limits of acceptable variations are 76.4% (the maximum one) and 23.0% (the minimum one).

Taking into account the obtained T/C values, the product of the T/C values in the first two tests is 0.19 and the product of the T/C values in the three tests is 0.083.

The successive investigations of the antitumoral pharmacotherapeutic effect of the PA_3 preparation on Walker 256 carcinosarcoma has conditioned the results included in table 4.

Table 4

Testing and retesting of the cancerostatic effect of the PA_3 polyphenolic preparation treatment (45mg/kg.b.w./i.p./daily) on rats bearing Walker 256 carcinosarcoma. Figures in brackets indicate the number of animals.

Group/ Treatment	M.T.W. (g)	% M.T.R.	T/C value	Statistical significance
CONTROL	15.2±1.0(15)	—	—	—
PA_3	8.8±1.4(10)	42.1	0.58	$p<0.002$
CONTROL	12.2±1.5(15)	—	—	—
PA_3	6.6±1.1(10)	46.0	0.54	$p<0.01$
CONTROL	14.6±1.3(15)	—	—	—
PA_3	8.1±1.3(10)	44.5	0.55	$p<0.002$

In the initial experiment, the induced antitumoral action —expressed by a decrease of the MTW compared with the control group — was illustrated by the MTR (42.1%) and T/C (0.58) values. In this case the T/C × 100 value is 58%, permitting the calculation of the admissible variability range, with an upper limit of 105.6% and lower one of 31.8%.

The primary retest confirmed the cancerostatic potential of the PA_3 preparate, characterized by both MTR (46.0%) and by T/C ratio (0.54). The corresponding T/C × 100 value was 54% and the T/C values product of these two tests was of 0.31.

Near values of the evaluation indices of PA_3 inducing antitumoral action were registered in the second retesting, being of 44.5% (MTR) and 0.55 (T/C ratio). The T/C × 100 value of the finale retest was 55%.

The product of the T/C values corresponding to all successive tests was of 0.17.

DISCUSSION

The discovery of a new cancerostatic agent and its use in the antineoplastic chemotherapy is the result of a complex process of preclinical and clinical research.

The chemotherapeutic programs of multistage preclinical screening, conceived to identify new active cancerostatic substances, foresee: numerous, successive and interdependent steps of investigation; qualitative and quantitative evaluation indices of specific pharmacodynamics effect; appreciation criteria of the induced antitumoral action (1 — 6), (8 — 10).

The preclinical characterization of a substance as antineoplastic agent requires:

— on the one hand, the evidence of its cancerostatic action and the demonstration of the pharmacotherapeutical effect reproducibility on adequate experimental models — objectives of the qualitative pharmacological evaluation — and,

— on the other hand, the appreciation of the antitumoral therapeutic effectiveness by: the establishment of the dose-response relationship; the comparison of the new drug activity on the tumoral development process with that of a standard cancerostatic agent of clinical use; the recording of a significant effect on tumors with different degrees of development — objectives of the quantitative pharmacological evaluation (6), (12), (13), (14).

The evidence of the antitumoral pharmacotherapeutic effect of the PA_2 III and PA_3 polyphenolic preparates, on two of three experimental tumoral systems used by us in testing 7, has imposed successive tests with these products in order to prove the reproducibility and stability of their specific action — a major problem of the pharmacological qualitative evaluation. This second stage, which implies a succession of three tests, must be performed in conditions identical with those of the initial test which has evidenced the antineoplastic pharmacotherapeutic action of the polyphenolic preparations.

The *in vivo* preclinical screening programs — used by us for the interpretation of the obtained data significance — established specific

criteria for the appreciation of the reproducibility of induced antitumoral action. Thus, the program of the Institute of Microbiology and Experimental Therapy from Germany requires, for the successive testing step, the registration of some close values of the induced MTR (3). The program of the National Institute for Cancer Chemotherapy from U.S.A. (5), which admits that the T/C ratio variance in different tests is an inherent consequence of the experience animal response variability, proposes another appreciation criteria :

— inclusion of the retest T/C × 100 values between superior and inferior limits of the admissible variability range ;

— the product of the T/C values of first two tests must be of 0.20 — 0.24 ;

— the product of the T/C values in all three tests must be of 0.08 — 0.09.

In the light of the above values, our results, obtained after the successive testing of the PA_2 III and PA_3 antitumor action on rats bearing either Guérin T-8 lymphotropic epithelioma or Walker 256 carcinosarcoma, can be discussed and interpreted.

The significant values of the mean tumoral regression (50.8%, 54.7%, 49.4% and 58.2%, 53.9%, 55.7%, registered on Guérin T-8 tumor bearing rats subjected to the antitumoral treatment with the polyphenolic preparates PA_2 III and PA_3, respectively ; 50.0%, 47.3%, 48.7% and 42.1%, 46.0%, 44.5%, induced on Walker 256 tumor by the therapy with PA_2 III and PA_3, respectively), obtained in the successive tests, are very close and greater than the imposed minimum value (35.0%).

The T/C × 100 values of retests (on Guérin T-8 tumor : 45.0%, 51.0% and 46.0%, 44.0% ; on Walker 256 tumor : 53.0%, 51.0% and 54.0%, 55.0%, calculated on the basis of the T/C ratios characteristics to that submitted to the cancerostatic therapy with the PA_2 III and PA_3 polyphenolic preparations) are situated between the upper and lower limits of the corresponding admissible variability ranges (89.2% — 26.9% and 76.4% — 23.0% ; 91.0% — 27.5% and 105.6% — 31.8%).

The T/C products — both of the first two T/C ratios and of the all T/C ratios —, estimated after the PA_2 III and PA_3 antitumoral treatment (on lymphotropic epithelioma : 0.22 and 0.11 as well as 0.19 and 0.083 ; on carcinosarcoma : 0.26 and 0.13 as well as 0.31 and 0.17), are either corresponding or near to the standard values, established by the reference American program.

The comparative analysis of our values with those stipulated by the preclinical screening programs for this second step of the qualitative evaluation certifies the reproducible and stable character of the antitumoral pharmacodynamics effect, induced by PA_2 III and PA_3 polyphenolic preparates.

Also, the results pointed out the cancerostatic pharmacotherapeutic spectrum of the studied preparates, highlighting at the same time the greater therapeutic effectiveness on the Guérin T-8 lymphotropic epithelioma.

Demonstration of the antineoplastic action reproducibility of PA_2 III and PA_3 polyphenolic preparations completes the qualitative

evaluation of this pharmacologic effect, conditioning the appreciation of the pharmacotherapeutic efficiency significance by the quantitative evaluation of the PA_2 III and PA_3 cancerostatic action.

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EFFECT OF THE ELECTROMAGNETIC RADIATIONS IN THE VISIBLE DOMAIN UPON THE DYNAMICS OF THE MEMBRANE POTENTIAL

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KCl irradiations in an electromagnetic field from the visible domain (546 nm) induces modification of the specific action of the substance upon the membrane potential, depending on the duration of irradiation. With 2 and 9 sec irradiation of the KCl, the cell membrane responds through a slower and weaker depolarization, as well as by a more rapid repolarization, as compared to the response given to the action of non-irradiated substance. In the case of 3, 5 and 11 sec irradiation, an ampler and more rapid depolarization occurs, and a slower repolarization of the cell membrane, as well.

The value of the resting potential of the cell membrane depends on the flux and the concentration gradient of K^+, increase of extracellular K^+ concentration inducing membrane depolarization (2 — 4), (8). The development of various biological phenomena is influenced by several factors, electromagnetic radiations included (5), (9), (11), (12). Nevertheless, the effects of such radiations upon the membrane potential, especially those from the visible domain (3) have been less studied.

The present paper discusses the dynamics of cellular membrane depolarization through an increased external K^+, under the influence of a preceding irradiation of the KCl from the Ringer solution with electromagnetic radiations in the visible domain (546 nm, 5000 lx).

MATERIALS AND METHODS

The experiments were performed on frog (*Rana ridibunda* Pall) sartorius muscle in normal Ringer solution (NR) containing 2.5 mM non-irradiated KCl and also in solutions with an increased K^+(30 mMKCl), non-irradiated (N) or irradiated (I) substance, for 2, 3, 5, 7, 9 and 11 sec (546 nm, 5000 lx). The membrane potential (MP) has been determined by technique of glass intracellular microelectrodes. Each experimental series was performed on 5 animals, following, in each case, the effect of the iradiated substance on a muscle, while following, on the pair muscle, the effect of the same concentration of non-irradiated substance (control sample = N), and afterwards comparing the results obtained. The data have been calculated by the Student test.

RESULTS

The results obtained evidence the fact that KCl irradiation has induced modifications of the cell membrane reactivity, with regard to both rate and amplitude of the KCl depolarization, and to the returning of the

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MP to its initial normal value (NRP), when washing the muscles with NR containing non-irradiated KCl.

The response of the cell membrane to the action of irradiated KCl is variable, depending on the irradiation duration. Thus, during a 2 sec. irradiation (Fig. 1), a decrease of the rate and amplitude of depolari-

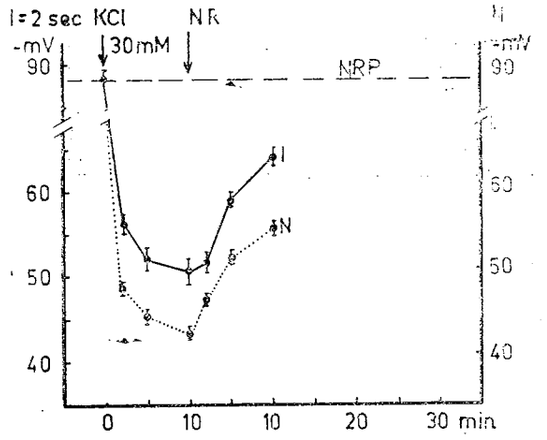

Fig. 1. — Membrane depolarization by 30 mM KCl, at a 2 sec irradiation (I). (N = non-irradiated = control; NR = normal Ringer; NRP = normal resting potential).

zation was recorded, accompanied, however, by a more rapid repolarization. Similar phenomena, although weaker, have been recorded with a 9 sec irradiation (Fig. 5-A).

In the case of a 3 sec (Fig. 2), 5 sec (Fig. 3) and, especially, 11 sec (Fig. 5-B) irradiation, an increase of the rate and amplitude of the mem-

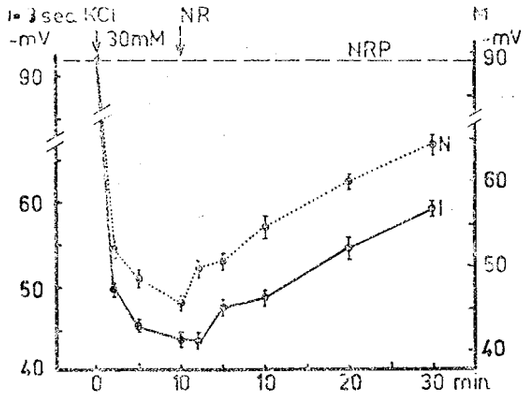

Fig. 2. — Membrane depolarization by 30 mM KCl at a 3 sec irradiation. Other explanations — see fig. 1.

brane depolarization was evidenced, along with a delay of its repolarization, as compared with the experiments performed with non-irradiated substance. At 7 sec irradiation, no differences have been observed between the action of the irradiation substance, as compared with the non-irradiated one (Fig. 4).

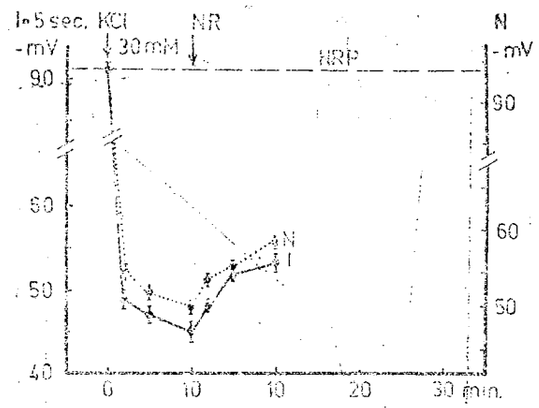

Fig. 3. — Membrane depolarization by 30 mM KCl at a 5 sec irradiation. Other explanations — see fig. 1.

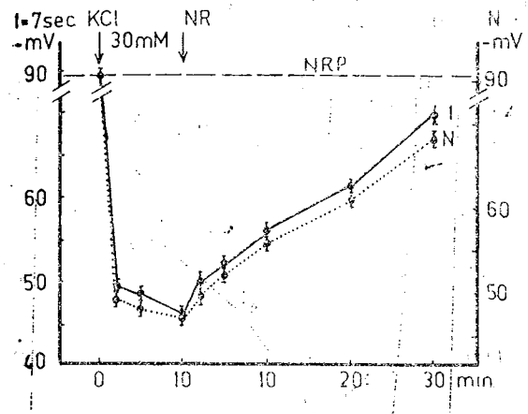

Fig. 4. — Membrane depolarization by 30 mM KCl at a 7 sec irradiation. Other explanations — see fig. 1.

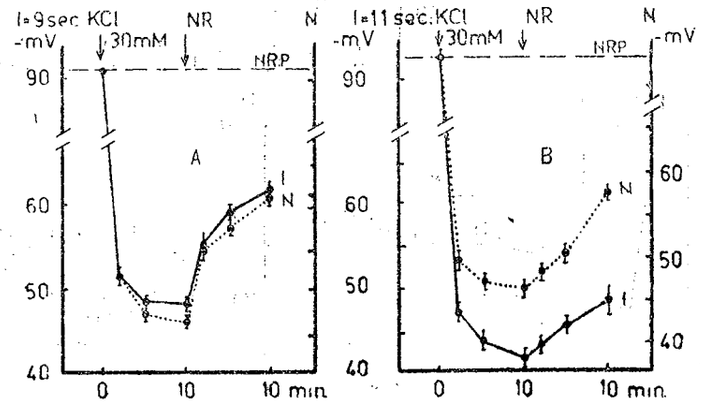

Fig. 5. — Membrane depolarization by 30 mM KCl at a 9 sec irradiation (A) and 11 sec (B) irradiation. Other explanations — see fig. 1.

as in the interaction between the cations' hydratation water and certain groups from the membrane molecular structure, under the action of the electromagnetic field (12).

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THE CHROMATOPHORES DISPOSING IN LIGHT AND DARK TEGUMENT OF SALAMANDRA SALAMANDRA (L.)

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The *Salamandra s.* tegument exhibits two types of differently colored areas : light and dark.
The light area — yellow-orange — is due to the xanthocytes which are dispersed in superficial derm(lax) and also among the basal and granulosum layers of epidermis. Below them, there are the iridocytes.
The dark area — black — contains melanocytes in lax derm, or insinuated among keratinocytes. The keratinocytes of basal and granulosum layers are loaded with melanosomes.
The tegumentary chromatophores (xanthocytes, iridocytes and melanocytes) do not have desmosomes.
The tegument of *Salamandra s.* has no dermal chromatophoric units.

INTRODUCTION

The color of lower vertebrates tegument is due to the chromatophores which contain specialised organits. The wide range of colors in these animals occurs because of the arrangement of three types of chromatophores : xanthocytes, iridocytes and melanocytes. On the other hand, this phenomenon is also due to the keratinocytes capacity to phagocite melanosomes (1, 6, 7, 11, 17).

In this paper we describe the ultrastructural differences between the dark and yellow-orange areas of *Salamandra s.* tegument.

MATERIAL AND METHODS

Small fragments of Salamandra s. tegument were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate, overnight (at 4° C) and then embedded in epon, cut on a Reichert OMU-3 ultratome, double stained with uranyl-acetate and lead-citrate. Electronphotomicrographs were taken on a Jeol 100 B electron microscope.

RESULTS

THE DISPOSING OF XANTHOCYTES IN SUPERFICIAL DERM LAYER, IN LIGHT AREAS OF TEGUMENT

Because the chromatocytes are dispersed in the derm and between epidermal cells, first of all it is necessary to describe the structure of mainly tegument's layers.

The cells of basal layer exhibit in basal pole unequally processes which penetrate superficial derm (fig. 1). These processes are strictly pursued by basal membrane and exhibit hemidesmosomes in which tonofilaments are anchored (fig. 1).

Immediately under the basal membrane a dense layer can b observed made of collagen fibres, dispersed in all space directions : unde this layer there are chromatophores.

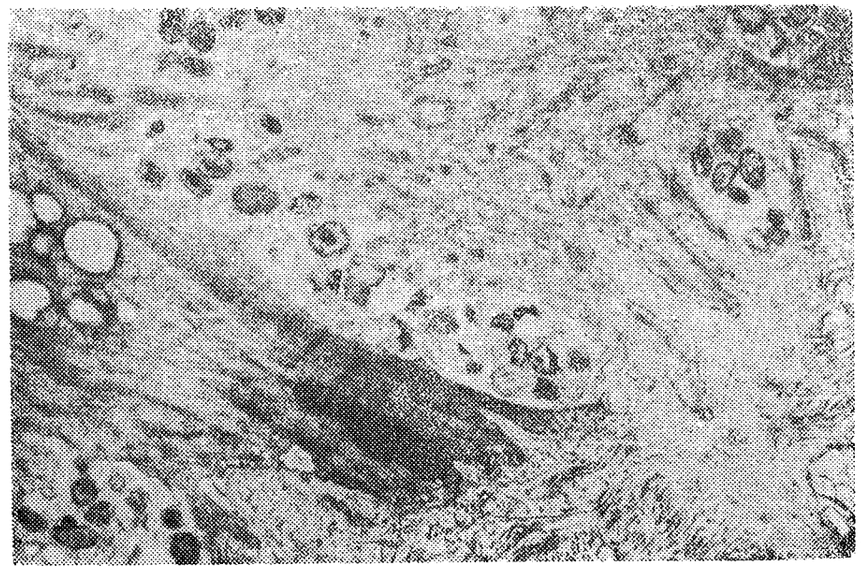

Fig. 1. — *Salamandra s.* tegument ; it can be observed that basal layer cells penetrate superficial derm in which there are bundles of collagen fibres. In the spaces between basal layer cells xanthocytes processes can be observed which contain pterinosomes.

The cells of the basal and granulosum layers are linked to eac other by desmosomes processes. Between these processes there are wid clear spaces in which the processes of xanthocytes are insinuated (fig. 2

The xanthocytes presence inside of basal layer cells is distinctiv for the yellow-orange areas of the tegument. These cells send betwee keratinocytes coupled processes, numerous prolongations without des mosomes. Between plasmallema of keratinocytes and xanthocytes adhe rent structures can't be observed.

In the xanthocytes pericarions and in their processes too, their spe cialised organits-pterinosomes can be observed. These organits exhib variable structure which should be due to a stade of their life cycl The most frequent structures are those in which the pterinosome have a spherical or ellipsoidal electronodense contour and also, those wit a central electronodense mass which is radially linked to the peripherica membrane.

In these areas of the tegument, immediately under superficial de mic layer, there are iridocytes (Fig. 3) which exhibit reflectory plates o different sizes and disposed in all space directions ; they have a side wit membrane and another one, in direct contact with the cytoplasm (Fig. 3

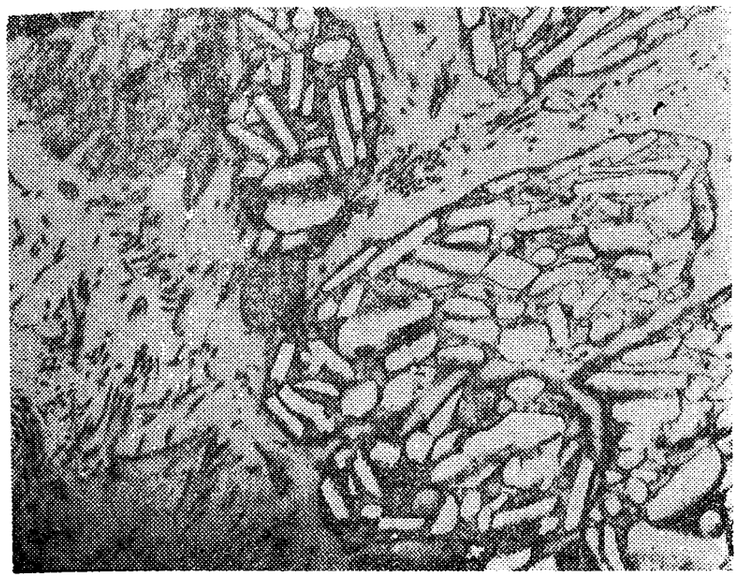

Fig. 2. — *Salamandra s.* tegument ; between granulosum layer cells there are xanthocytes processes the plasmalema of which have no desmosomes.

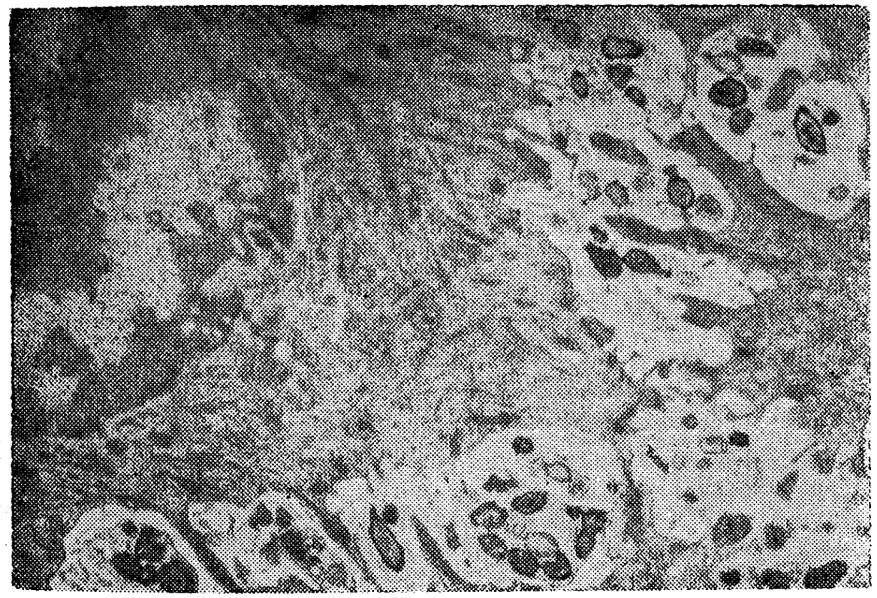

Fig. 3. — *Salamandra s.* tegument ; iridocytes processes can be observed which are loaded with disorderly disposed reflectory plates. A side of these exhibits electronodense membrane and other one comes in direct contact with the cytoplasm.

THE DISPOSING OF MELANOCYTES IN SUPERFICIAL DERM LAYER AND BE TWEEN KERATINOCYTES, IN DARK AREAS OF THE TEGUMENT

As in light areas, basal layer cells also exhibit inequally processe which penetrate the derm ; they also have hemidesmosomes and the basa membrane pursues them. Under basal membrane there are thick packet of collagen fibres.

The basal layer cells have tonofibrils, prekeratinosomes and othe characteristic cellular organites. Between and inside basal layer cell melanocytes processes can be observed loaded with melanosomes, whic fill the whole cytoplasm (Fig. 4). There is a difference between melano

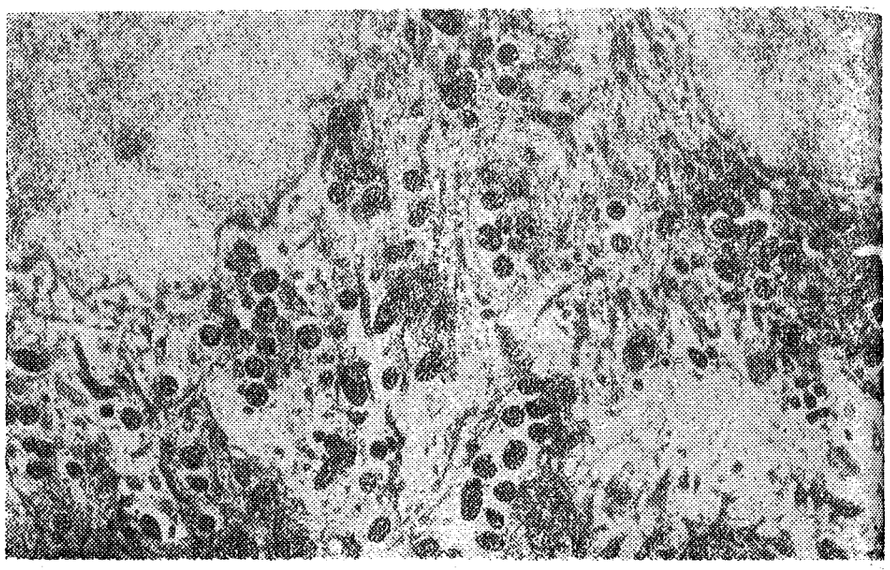

Fig. 4. — *Salamandra s.* tegument ; fragments of two basal layer cells in black area. The cytoplasms is loaded with melanosomes looking like those in melanocytes processes.

somes in melanocytes and those from keratinocytes. The keratinocyte melanosomes lie in clear spaces-like the vesicles-with variable sizes. In some cells, melanosomes are very few or even absent (fig. 5). Besides the granulation of low electronodensity and variable sizes can be observed. In such cells, these granulations represent prekeratinosomes or keratinosomes in different stages of elaboration.

In the horny layer vicinity, these granulations get to have the same electronodensity with that of tonofilaments. Both are ready to become homogene like horny layer (fig. 5). Melanosomes conserve their integrity even in superficial horny layer (fig. 5).

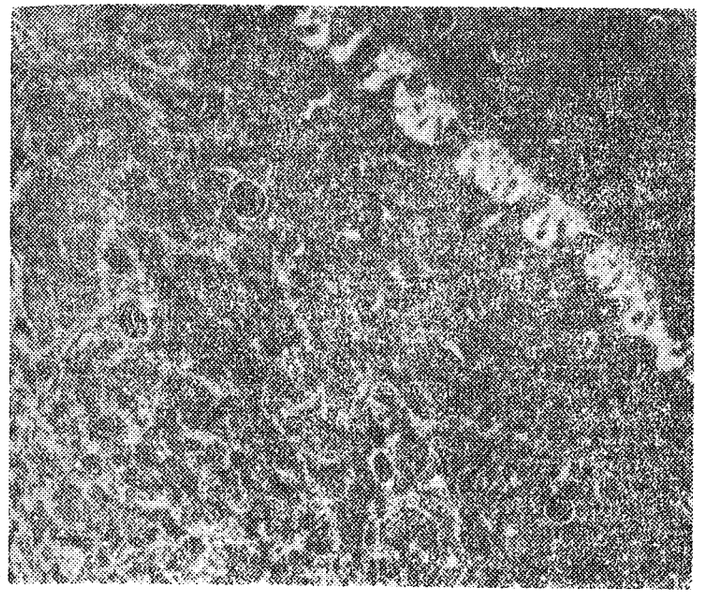

Fig. 5. — *Salamandra s.* tegument ; section in horny superficial and granulosum layers ; among keratinosomes some melanosomes can be observed.

DISCUSSIONS

The tegument color depends of the arrangement of those three chromatophore types (xanthophores, iridophores and melanophores). The yellow-orange color in Salamandra s. tegument is determined by a superficial disposing in the derm and also in the basal layer cells of xanthophores.

Immediately under the epidermis there is a dense layer of collagen fibres and only under this, lie the iridocytes. Generally speaking, immediately under epidermis there is a lax derm in which, in other vertebrates (fishes, amphibians, reptiles) chromatocytes may organize dermal chromatophoric units, like in *Anolis carolinensis* (1, 17).

The reflectory plates in iridocyte exhibit a side with thick electronodense membrane, and the other one does not have a membrane, so its electronoclear content is in direct contact with the cytoplasm (6, 7, 1, 14). This structure sugests that reflectory plates are made of nuclear membrane, as we demonstrated in the Natrix natrix iridocytes (11).

In the black areas of the Salamandra s. tegument, the melanocytes are situated immediately under epidermis and also among basal and granulosum layers cells. In these areas there are melanosomes even in keratinocytes where, sometimes they fill a big part of the cell cytoplasm. Sometimes, the melanosomes are conserved even in those cells which are in a very advanced stage of keratinisation or completly keratinised, like in external horny layer cells.

We could not observe a melanisation process in keratinocytes, as there is in epidermis of high vertebrates. We think that it is not only a phenomenon of phagocitosis of melanocytes processes by keratinocytes, but also of a melanisation phenomenon. The melanosomes' existence in all epidermal cells and in the melanocytes of these zones too, confers the dark color of the tegument; in the deep derm there exist other cromatocyte types (iridocytes and xanthocytes) as well.

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ULTRASTRUCTURAL ASPECTS OF APOPTOTIC PROCESS OF PREIMPLANTATIONAL BLASTOCYST

DORINA MIRANCEA and N. MIRANCEA

In this paper we present some ultrastructural aspects which characterize the apoptotic process (programmed cell death) of the preimplantational embryos of *Sus scrofa domestica* used in embryotransfer biotechnology. During electron microscopic investigations concerning cytodifferentiation of primary cell lineage, we have noticed the presence of some cells from the inner cell mass (ICM) having the intercellular junctional streuture of desmosomal type partially degraded, concomitantly with the mitochondrial condensation and intracytoplasmic myelinic figures foundation which suggest early debut of some cell degradation in embryo development. Nuclei of the cells undergoing ultrastructurally modifications which are generally characteristic to apoptotic cells but, exhibit a peculiar way of passing the nuclear material from the nucleus into the cytoplasm. One of the first nuclear changes is focal enlargement of the perinuclear space between internal and external nuclear envelope. Subsequently, nuclear pores along dilated envelope are degraded and large quantities of fibrilogranular nuclear material are extruded into dilated perinuclear space. Finally, internal nuclear envelope appears discontinuous while external membrane becomes highly convoluted while the nuclear material penetrates into the cytoplasm.

Under normal conditions a primary consequence of cell specialization is the inevitable death of somatic cells. The time sequence and the circumstances under which death occurs, however, are different in the different tissues (12), (17). Cell death may follow two distinct morphological and biochemical patterns: (a) necrosis and (b) programmed cell death (apoptosis).

Cell degeneration by necrosis is a pathological event which occurs as a consequence of severe stress conditions: physical factors (mechanical actions, high energetic radiations etc.), toxic agents, viral or bacterial infections, ischemia or hypoxia etc. Necrotic cells lose their synthetic functions and mitochondria are dilated. The cell volume increase is reversible in its early stages by a severe cell oedema but is followed by irreversible damage to cell and organelle membranes so that the cell can be stained with vital dyes such as Trypan blue (18). Finally, some plasmamembrane domains, chromatin and cell organelles are destroyed (cell lysis) and cell content will be delivered which induces the inflammation process and phagocytosis.

By contrast, cells engaged in a suicide " programme (programmed cell death) preserve their biosynthetic capacity and are metabolically active. Usually, apoptotic process affects isolated cells (individual removal of a cell from tissues). The cells stimulated to enter apoptosis process in their way to die pass through a series of morphological and biochemical stages (3). In the intial phase, an individual cell, embedded in normal tissue, loses contact with adjacent cells (Willie A. H., et al., 1980, cited by Fesus L., et al. 1991), (16) and cell organelles are relative unaltered

(3), (18) while endoplasmic reticulum can be dilated (3). Subsequently, chromatin is condensed (1) and then fragmentation of the cell into apoptotic bodies occurs which contain cellular remnants (Kerr J. F. R., et al., 1972 cited by Fesus L., et al., 1991), not stained with yital dyes (18). The phagocytosis occurs before cell lysis and no inflamation response is induced (3), (18).

Control of cell population by programmed cell death is used for in eliminating cells that are requiered for a limited time only (11), (18). Programmed cell death plays important roles in a variety of situation including separation of the digits, fusion of the palate, regulation of T cells in the thymus, self-destruction of excess of neurons, involution of the prostate after castration, during keratinocyte maturation (9). Programmed cell death also occurs during embryogenesis (10), (18).

In previous papers (6 — 8) we reported on electron microscopic investigations regarding some ultrastructural aspects (cytodifferentiation, dynamics of intercellular relations, cytoskeleton and intercellular junctions development) as successive cell events appearing in pig preimplantational embryos used in embryotransfer. In this paper, we present some ultrastructural aspects which characterize the apoptotic cells during preimplantational embryogenesis of *Sus scrofa domestica*.

MATERIALS AND METHODS

The embryos (2, 4, 8, 16 blastomeres, morula and blastocyst were surgically obtained from *Sus scrofa domestica* females submited to a follicle-stimulating treatment with serum-gonadotrophin i.v. 1,500 I.U., followed three days later by i.v. 750 chorionic-gonadotrophin. The embryos were fixed in 2.5% Glutaraldehyde and 2%Osmium tetroxide, then embedded in Epon. The ultrathin sections contrasted with uranium acetate and lead citrate were examined by means of a Philips electronmicroscope at 50 kV.

LEGEND OF FIGURES

Fig. 1. — In the interblastomeric spaces (IS) numerous microvilli belong to blastomeres (B_1 and B_2), different as dimensions and vacuum electronmicroscopic vacuoles (v) as well as an amorphous electrondense substance could be seen (asterisk) (× 5,200).

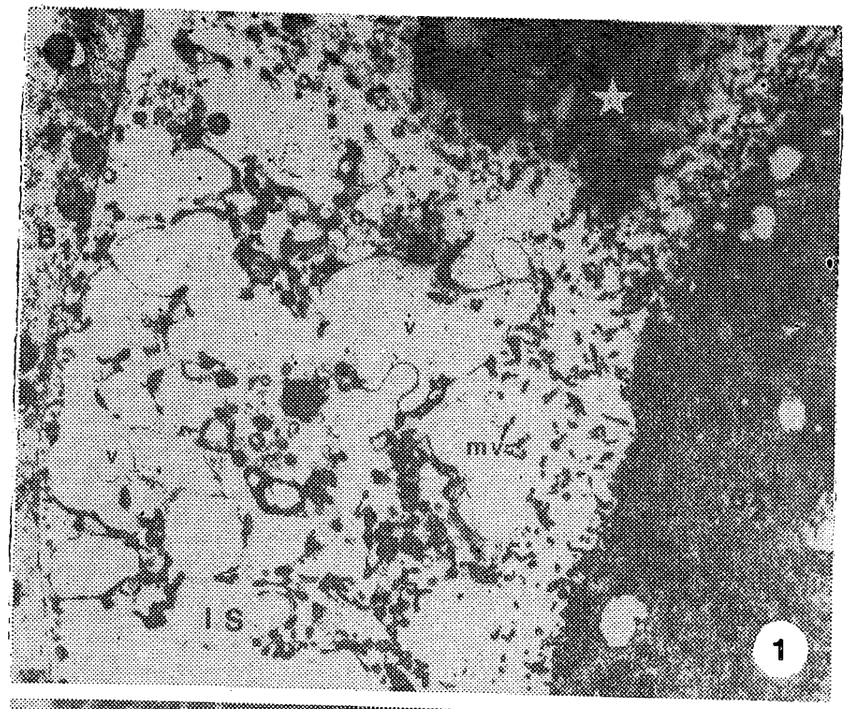
Fig. 2. — A remnant of blastomeric material surrounding by individual membrane (arrow) is detached from blastomer (B); microvilli = (mv). (× 19,500).

Fig. 3. — Desmomal junctions between two adjacent embryoblastic cells (EC_1 and EC_2) with characteristic elements well represented : dense attachment plaque (curved arrow) and intercellular material (Δ) (csk f) = cytoskeletal filaments (× 70,000).

Fig. 4. — Extensive intercellular junctions type (D_1 and D_2) strong connect two embryoblastic cells (EC_1, EC_2). In addition to desmosomes D_2 a "zonula occludens" (zo) can be seen (× 60,000).

Fig. 5. — Two blastomeric sectors (B_1 and B_2) emit microvilli (mv); sometimes they could be distribuited as bundles (white (squares) directed towards blastocellian liquid (Blc). Numerous and polymorphic vacuoles (v) there are in the cytoplasm of blastomeres. csk f = cytoskeletal filaments. (× 10,500).

Fig. 6. — A sector of blastomer (B) washed by the blastocellian liquid which emitt microvilli directed towards blastocellian cavity and even cilia (star) cross sectioned can be detected. (× 26,000).



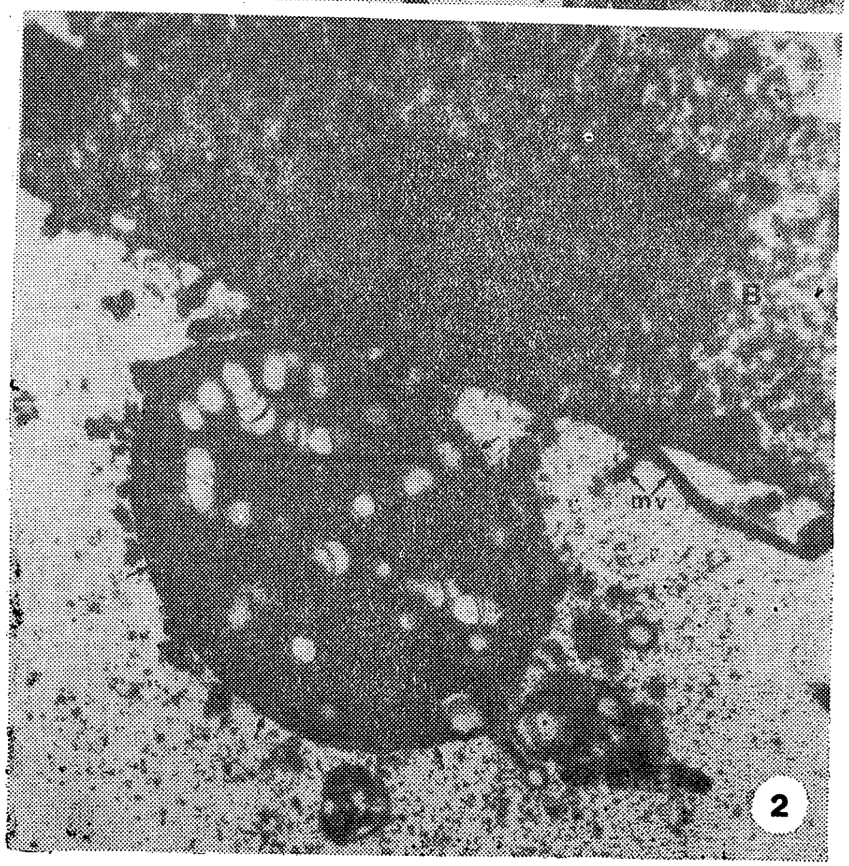
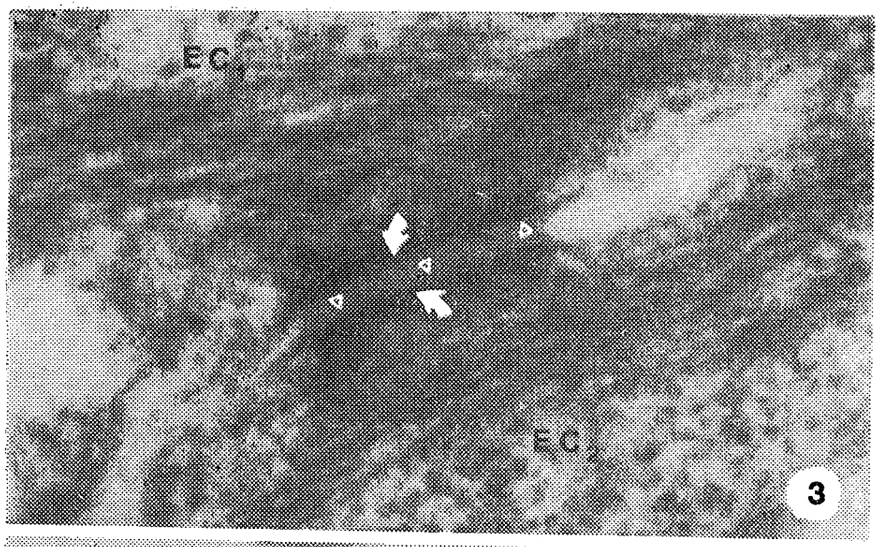
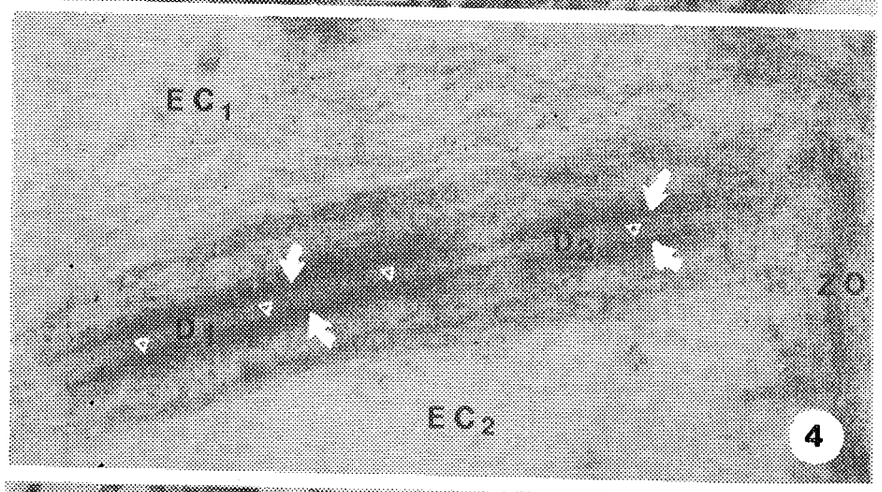
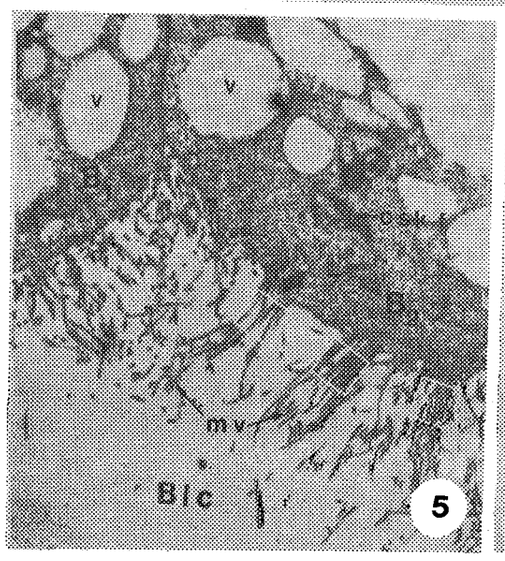


Fig. 1, 2







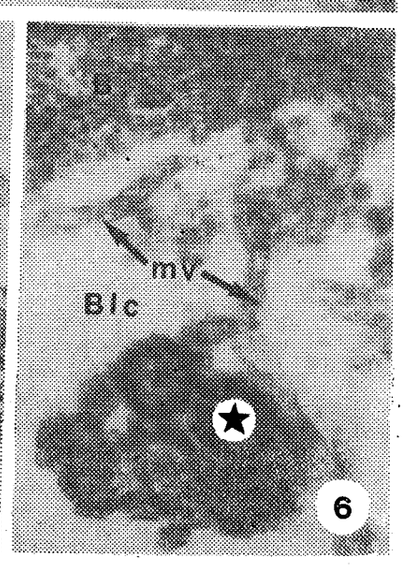
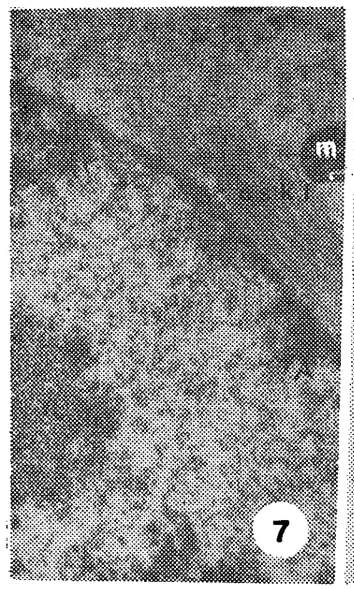
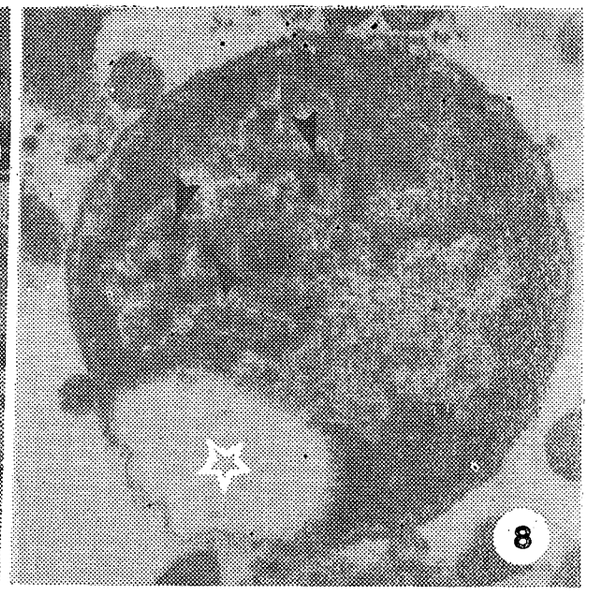
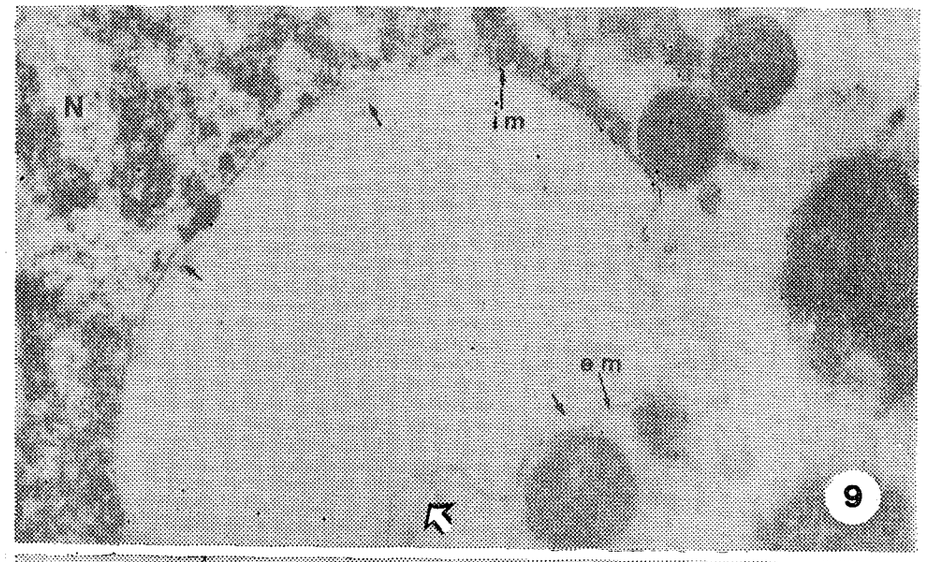


Fig. 3 — 6







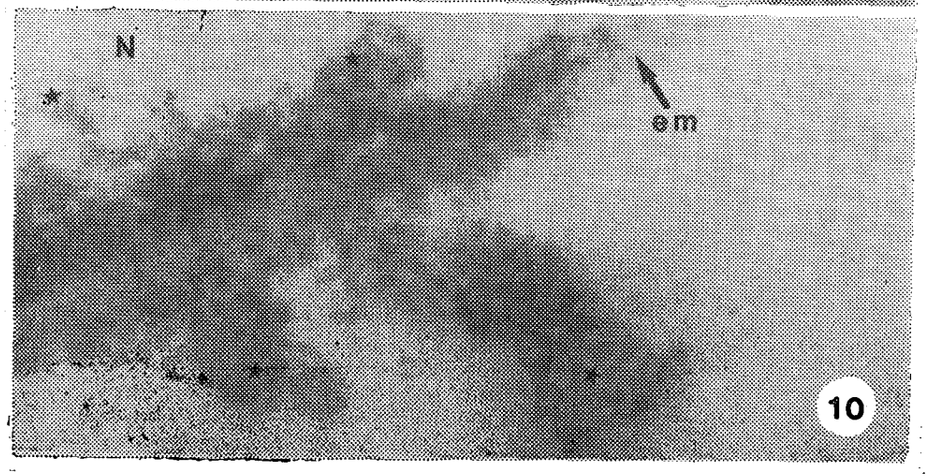
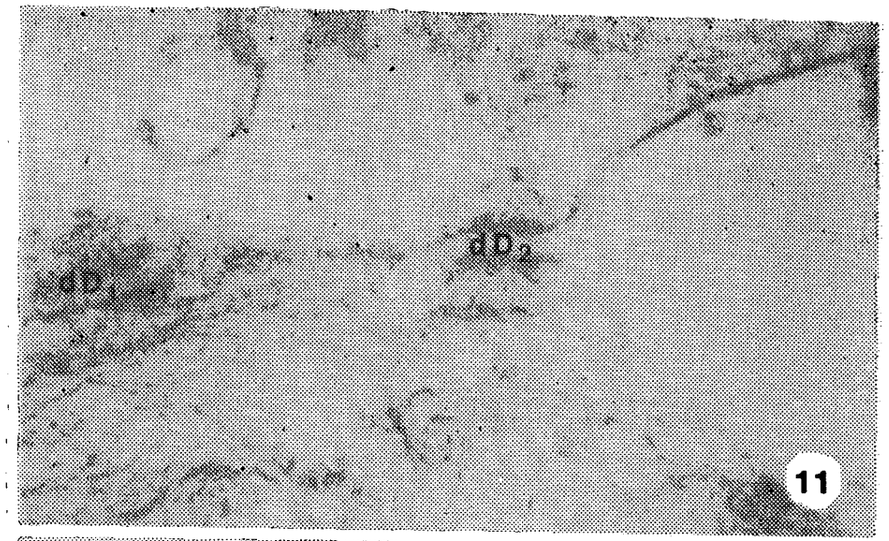
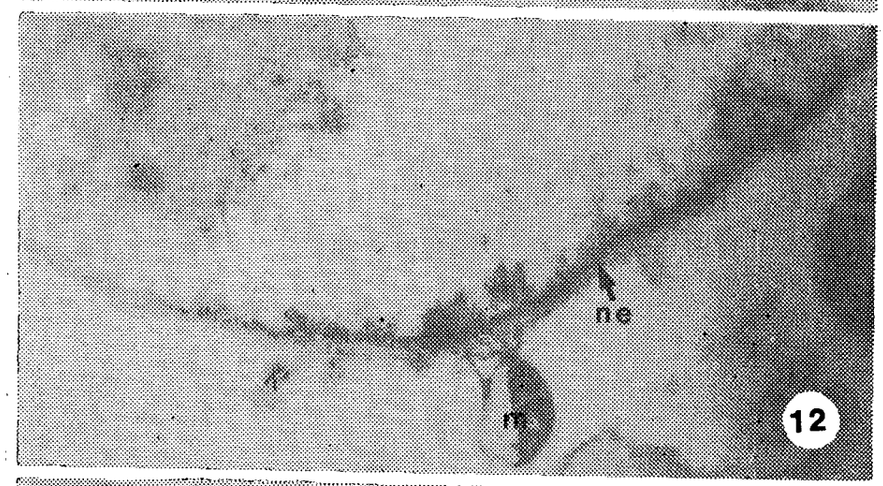


Fig. 7 — 10





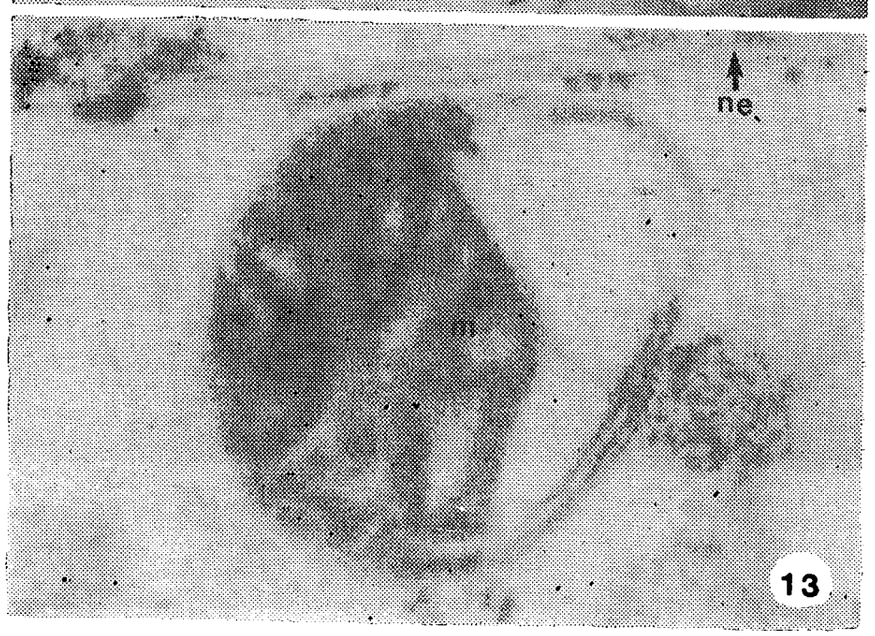


Fig. 11 — 13

RESULTS AND DISCUSSIONS

Snow M.H.L., (1987) considers that there are four stages when cell death occurs and plays a role in development : (a) in the inner cell mass of blastocyst during implantation ; (b) in the epiblast during amniotic cavitation formation ; (c) in the epiblast/ectoderm during mesodermal formation ; (d) in the primitive endoderm.

Programmed cell death occurs very early in the embryo development. By apoptosis, in the blastocyst, defectively differentiated cells which might interfere with the development. By distroying some cells of the inner cell mass (ICM), it may maintain appropriate ratio of embryoblastic cells and trophoectoderm (10).

During the first and second mitotic divisions (clivage) of *Sus scrofa domestica* zigot a huge elimination of cellular material in the interblastomeric space could be seen. The extrusion of cytoplasmic material in the intercellular space precedes the nuclear material extrusion (Figs 1 and 2).

Checiu M., et al., (1990) have been demonstrated by microcinematography that cell divisions of mouse preimplantational embryos were preceded by a visible activation of cell surface, by wavy mouvement of cell surface and emition of cellular protrusions. The rearrangement of blastomeres with their topographic relationship change was also detected. It seems that a very active measurement of the blastomeric surface detected by microcinematography as a "ebullition" corresponds to the moment when partial cell content is eliminated in the interblastomeric space which we detected during electron microscopic investigation of pig embryos.

Numerous vacuoles and lipid droplets can be seen inside the blastomeres (Fig. 1 and 2). In the vicinity of secondary lysosomes osmiophilic vacuoles can be detected. Sometimes, near the blastomeres a huge vacuole filled with a substances similar to cytoplasmic contentent could be observed (Fig. 2).

After blastocellian constitution, the desmosomal junctions are numerous and extensively developed (Figs. 3 and 4) and they strongly

Fig. 7. — Ultrastructural aspect of a nucleus belongs to normal embryoblastic cell : the euchromatin is prevalent : scanty heterochromatin (open arrow) is adherent to the internal membrane of the nuclear envelope ; (m) = mitochondria. (× 18,600).

Fig. 8. — Ultrastructural aspect of the nucleus belongs to the beginning of apoptotic cell. On extensive areas, nuclear material is granular-condensed (arrowhead). Perinuclear space (star) is unifocal dilated. (× 9,000).

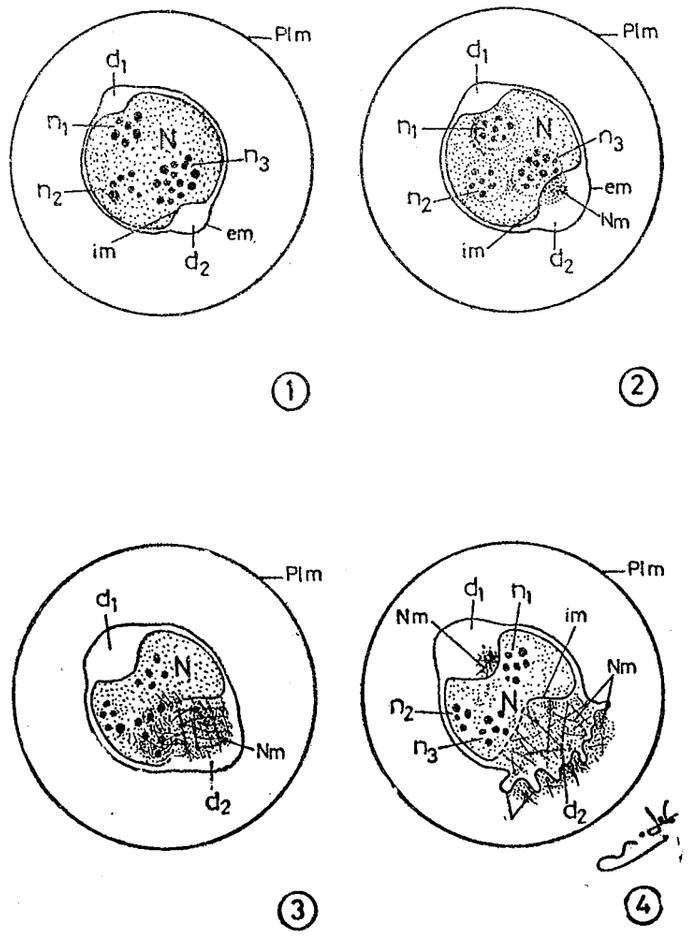
Fig. 9. — In the perinuclear space excessively dilated (star) of an advanced stage apoptotic cell there is an fibrillo-granular material passing from nucleus (N) through internal membrane (im) of the nuclear envelope still preserved and then pass through external membrane (em) of the nuclear envelope to be intracytoplasmically delivered (open arrow). (× 17,200).

Fig. 10. — Ultrastructural detail to point out nuclear material (asterisk) during their passage from the perinuclear space towards adjacent cytoplasm to external membrane (em) of the nuclear envelope. (N) = nuclei. (× 50,700).

Fig. 11. — Two apoptotic cells in way to be desconnected by desmosomal degradation (dD_1 and dD_2). (× 53,200).

Fig. 12. and 13. — Often, in the apoptotic cells, mitochondria (m) are adjacent to nuclear envelope (nc). Most mitochondria are partially vacuolised concomitantly with condensation. (Fig. 12. × 16,800) ; (Fig. 13. × 45,300).

connect adjacent cells. Numerous intermedium filaments cytoskeleton of the cells are inserted into dense cytoplasmic plaques.



Scheme no. 1

Schematic representation of succesive ultrastructural aspects concerning nuclear material extrusion through bifocal dilatation of perinuclear space of some apoptotic embryoblastic cells (original)

Stage (1): Inition of bifocal dilatation of a perinuclear space (d_1 and d_2) neighbourhood of the nucleoli.

Stage (2): Progressive enlargement of perinuclear space and the debut of fibrillogranular nuclear material (Nm) extrusion into the cisternal space.

Stage (3): Massive extrusion of fibrillogranular nuclear content in the cisternal space.

Stage (4): Internal membrane (im) of nuclear envelope is partial discontinuous and nuclear content passed through numerous infoldings of external membrane (em) into the cytoplasm.

Pl = plasmamembrane; N = nucleus; $n_1 - n_3$ = nucleoli; d_1 and d_2 = dilatation of perinuclear space; im = internal membrane of nuclear envelope; em = external membrane of nuclear envelope; Nm = nuclear material during their passage towards cytoplasm.

During electron microscopic investigations on cytodifferentiation and primary cell lineage of suine embryos, we have remarked the existence of some cells from ICM with partially degraded intercellular junctions of desmosomal type in spite of the fact that the ultrastructure of the nucleus is still preserved. The degradation of some plasmamembrane domains (especially at the desmosomal site) concomitant with mitochondria condensation and intracytoplasmic myelinic structures foundation suggest debut of some cell degradation. Similar ultrastructural aspect was described in the mouse blastocyst (11) and that was considered as the consequence of programmed cell death as a preliminary request for normal development. Moreover, we observed that in pig blastocyst cells show a peculiar way of extrusion of the nuclear material into cytoplasm.

Our electron microscopic results demonstrated that nuclear material extrusion in the engaged cells under apoptotic process could be developed in this way (Scheme nr. 1 and Figs. 8 — 10): initialy uni- or bifocal dilation of the perinuclear space (maximum three focal points per nucleus could be detected) usually in the neighbourhood of the nucleoli. Perinuclear spaces progressively enlarge. Inner membrane centripetal push nuclear content so that nucleus achieves half-moon or biconcave lens shape. Nuclear content as fibrillogranular material could be seen into perinuclear space (Figs 8 and 9). Sometimes, internal membrane of nuclear envelope is partial discontinuous and nuclear content passes through the highly convoluted external membrane into the cytoplasm (Figs 9 and 10). Extrusion of nuclear material as result of genic amplification was described especially during Vertebrate ovogenesis as well as for zebra fish (*Brachydanio rerio*) (5). Ultrastructurally, the passage of nuclear content into ovocyte cytoplasme, compared with the extrusion of fibrillogranular material from apoptotic nucleus into cytoplasm is different: during ovogenesis the passage of ribonucleoprotein material from nucleus into cytoplasm is made throw nuclear pores (often, their number is multiplied in the nuclear sector implied in this process and the perinuclear space is not dilated, while apoptotic cell of preimplantational *Sus scrofa domestica* embryos has degraded nuclear pores and perinuclear spaces is excessively enlarged where nuclear envelope is implyied in passage of nuclear content.

Which are the aim and the mechanism(s) of the apoptotic process so early expressed in embryo development? Pierce G. B., et al., (1991) consider that ICM of early blastocyst has the potential to differentiate trophectoderm but programmed cell death may eliminate redundant pretrophectodermal ICM cells. Sinh V.P., et al., (1990) have demonstrated that by degradation of some ICM cells of *Cynopterus sphinx*, small proamniotic cavities could be constituted (debut of amniogenesis). We consider that the origin of the blastocellian cavity of *Sus scrofa domestica* embryos could be interblastomeric vacuoles early eliminated during embryogenesis. The large and clear electron microscopic vacuoles situated at the apical pole of embryoblastic cells adjacent to blastocelle could contribute to the blastocellian liquid, too. Numerous microvilli (sometimes, they could be distributed as bunches and even cillia could be directed into blastocellian cavity (Figs 5 and 6). Finally, apoptotic cells have varied degrees of degradation of the desmosomal junctions (Fig. 11) which leads to the new positional information acquisition. Often, in the apoptotic

cells, some mitochondria partial condensed and/or vacuolysed are in a contiguity relation with external membrane of the nuclear envelope (Figs 12 and 13).

Cell degradation followed by cell death in the pig embryo is a prerequisite for normal development? The intime mechanism of apoptotic process is not very well known yet but there are a lot of reports that intra- and extracellular factors could be responsible for this process. We underline the fact that during early embryo development of *Sus scrofa domestica*, the apoptotic process showed a peculiar aspect, exhibited by the massive extrusion of nuclear substance into cytoplasm, therefore of an informational material probably necessary for the biosinthesis of a factor which could contribute to the self cell-lysis, or of an extracelullar component whose biological significance in early embryogenesis we still ignore. Sophisticated equipment is necessary to solve this problem.

Ota et al., (1962, cited by Strange R., et al., 1992) have reported that during mammary gland involution, nucleic acid content of apoptotic cells is decreasing. Moreover, Fesus L. et al., (1991) consider that the apoptotic process requests metabolic energy and RNA and protein synthesis (4), (15).

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CORNEAL COLLAGEN FIBRILLOGENESIS *IN VITRO*

LUCIA MOLDOVAN *, ANCA OANCEA *, D. TURCU ** and OTILIA ZĂRNESCU ***

Our objective was to isolate type I and V collagen from mature bovine cornea and to study the interaction of these collagen types *in vitro* by electron microscopy. Type I and V collagen were obtained by limited pepsin treatment followed by salt fraction. In vitro, type I collagen formed fibrils with the characteristic 67 nm period, while type V collagen had no capacity to form striated fibrils. The aggregates obtained by mixing type I and V collagen seen by electron microscopy were fibrils with typical banding pattern and the average diameter much smaller.

Collagen molecules are synthesized, secreted into the extracellular space and organized into striated fibrils with a tissue-specific organization. Several studies have demonstrated the effect of a variety of factors on collagen fibril formation "in vitro". These include the interaction of different collagen types (1), (2), (3), the extent of procollagen processing (4) and the interaction with proteoglycans (5). The collagen types presented may be important in the determination of fibril architecture. Type I, type II and type III collagen form banding fibrils in tissues (6), while type IV collagen comprises networks in basement membranes (7).

On the other hand, the fibrils of many tissues are "heterotypic" structures, composed of different combinations of fibril forming collagen co-assembled (8), (9). For example, collagen type I and III are co-assembled in the dermis and the tendon (10), types II, IX and XI in cartilage (11), types I and II in the primary corneal stroma and types I and V in the secondary corneal stroma (12). It has been suggested that such a co-assembly of different collagens could determine properties of fibrils, for example fibril diameter (13). The mature bovine cornea is composed predominantly of type I collagen (84%), but also contains approximately 15% type V and type III less than 1% (14).

Biochemical and immunohistochemical observations have suggested that the interaction of type I collagen and type V collagen may regulate corneal fibril diameters.

Our objective was to isolate type I and V collagen from mature bovine cornea and to examine the interaction of these collagen types "in vitro" by electron microscopy.

MATERIALS AND METHODS

The bovine corneas from adult animals (2 years) were provided from the slaughter house.

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PREPARATION AND CHARACTERIZATION OF TYPE I COLLAGEN AND TYPE V COLLAGEN

The bovine corneas were finaly cut and incubated with pepsin (in 1/10 ratio) in 0,5M acetic acid, at pH 3,0 for two days, at 4°C. The digestion mixture was diluted 1 :1 with 0,5M acetic acid, and centrifuged for 40 min at 30000 × g in order to remove undigested tissue. The pepsin digest was precipited with 0,7M NaCl. At this concentration, type I collagen readily precipitates from solution, while type V collagen remains in solution. The collagen precipitation at this step was redissolved in 0,5M NaCl/0,05M TRIS-HCl, pH 7,5 and subsequently fractionated by increasing the NaCl concentration over the range of 1,1 to 4,5M NaCl as previously described (15), (16). The collagen remaining in solution (type V) was precipitated at 1,2M NaCl (16), (17), redissolved in 0,5M NaCl/0,05 TRIS-HCl, pH 7,5 and fractioned by dialysis versus 0,02M NaCl/0,01M TRIS-HCl pH 8,6 containing 2,0M urea. Then, the precipitate was redissolved in 0,5M acetic acid and dialyzed versus some solvent.

Purified collagens were characterized by SDS-polyacrylamide gel electrophoresis on 5% gels according to the Laemmli's modified method (18).

RECONSTITUTION OF COLLAGEN FIBRILS

Type I and type V collagen were mixed in ratio 5,5 :1. This collagen solution as well as type I and type V collagen only, were dialyzed versus PBS, at4°C with several changes. The dialyzates were incubated at 37°C for 8h.

ELECTRON MICROSCOPY

The dialyzates were dropped on carbon coated mesh grids and negatively stained with 1% phosphotungstic acid pH 7.0. Then, they were examined using a Jeol 7C transmission electron microscope.

RESULTS AND DISCUSSION

Type I and type V collagen were obtained from mature bovine corneas by a limited pepsin treatment followed by salt fraction.

The purity of the collagen recovered in the selective precipitation steps was evaluated by SDS-polyacrylamide gel electrophoresis. Collagen precipitated from the pepsin digest at 0.7M NaCl and the fractions of this collagen precipitating from neutral solution at 2.5M and 3.5M NaCl migrate similarly to a comparable sample of type I collagen from rat tail tendon. In contrast, collagen precipitated from the supernatant at 1.2M NaCl migrates similarly to a sample of type V (data not shown).

The in vitro formation of type I and type V collagen fibrils was studied using purified these types. The solutions of collagen were dialyzed at 4°C, versus PBS and incubated at 37°C, for 8h. Aggregates formed under these conditions in vitro were negatively stained and examined by electron microscopy. Electron micrographs (fig. 1A, 1B) show that type I collagen forms fibrils with the characteristic 67nm period, while type V collagen has not the capacity to form typical striated fibrils.

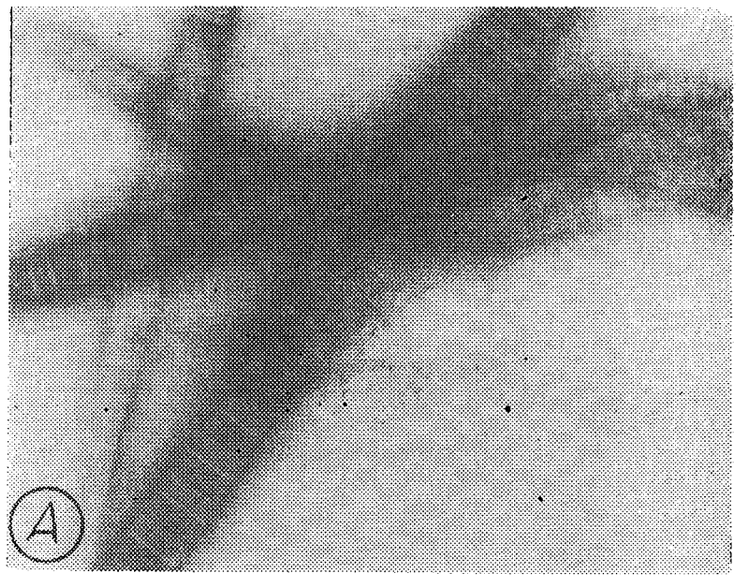
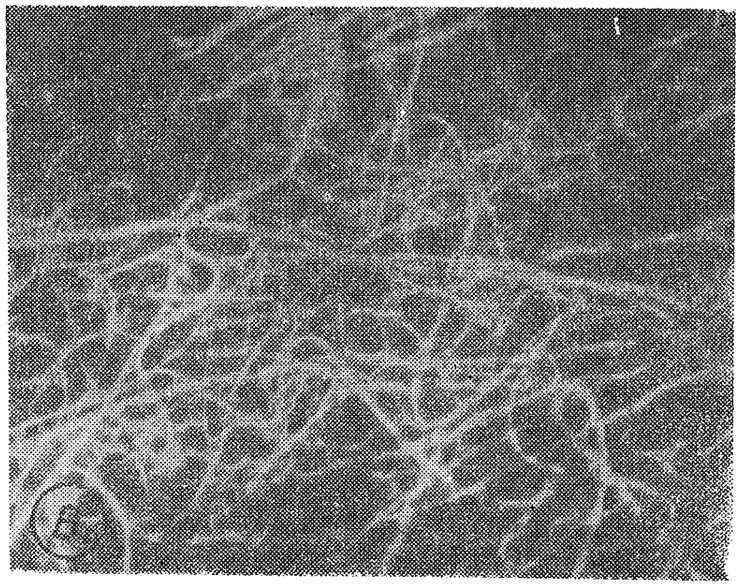


Fig. 1. — Electron micrographs of collagen fibrils from bovine corneal stroma. The bar corresponds to 330 nm.
(A) Type I collagen fibrils



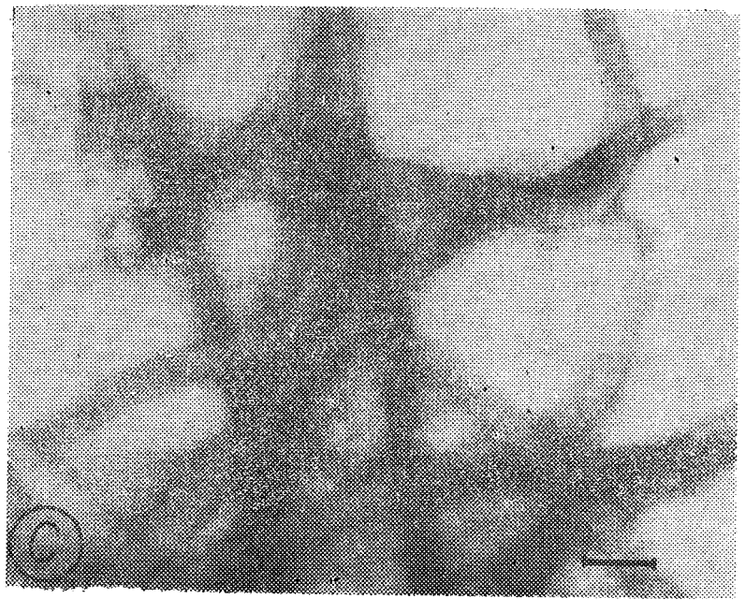


Fig. 1 (B) Type V collagen fibrils
(C) The mixture of type I and V collagen fibrils.

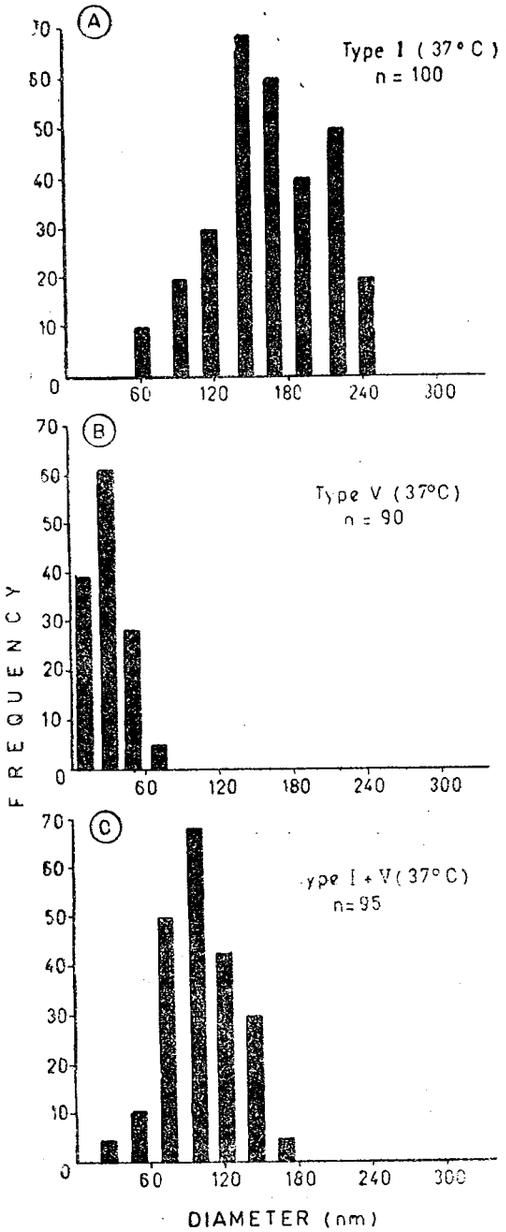


Fig. 2. — Distribution of diameters of type I and V collagen fibrils. (n — number of fibrils measured.)
(A) Type I collagen fibrils
(B) Type V collagen fibrils
(C) The mixture of type I and V collagen fibrils

On the other hand, the average diameter of the type V collagen fibrils is much smaller (fig. 2B), close to 30 nm; whereas the diameters of type I collagen fibrils (fig. 2A) are close to 150 nm.

To examine the interaction of these two different types of collagen during fibrillogenesis, the aggregates were formed by mixing type I collagen with type V collagen in ratio 5,5 :1 (as in tissue). The precipitates obtained, seen by electron microscopy were fine flexible fibrils, with typical banding pattern (fig. 1C). The average diameter of hybrid fibrils was smaller than 100nm (fig. 2C). This experiment demonstrated that the interaction of type V collagen with type I collagen decreased the diameters of fibrils formed in vitro.

The "in vitro" studies described by Adachi (19), also indicate that the collagen types I and V, obtained of human placentas, co-assemble to form heterotypic fibrils. Birk et al. (20) have suggested that type V collagen may have a regulatory role in the control of fibril architecture through its interactions with type I collagen.

Our results indicate that collagen type I and type V interact "in vitro", these interaction is responsible for the control of collagen fibril diameters. However, these fibrils are substantially wider than the fibrils of the corneal stroma "in vivo". "In vitro" assembly is not controled as the fibril-forming process "in situ", because cellular control of the mixing of different collagen types probably play an important role in the control of fibril formation (21).

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CHANGES OF THE STRUCTURE AND FUNCTIONING OF THE BENTHIC *OLIGOCHAETE* COMMUNITIES FROM THE DANUBE DELTA AQUATIC ECOSYSTEMS (1976–1982). 2. THE ASSESSMENT OF THEIR ROLE ON ECOSYSTEMS PRODUCTIVITY

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This paper is dealing with the investigation of some aspects regarding energy flow in five lakes of the Danube Delta during 1976–1982 interval. The parameters of energy budget, the assimilation efficiency (U^{-1}), the efficiency of utilization for growth of the assimilated energy (K_2), the coefficient of consumed food for growth (K_1) and the turnover rate of biomass (P/B) was determined. The productive potential of benthivorus fishes based on food supplied by the dominant oligochaete populations in different lakes was also estimated.
In these eutrophicated lakes, the trophic basis represented by the benthic oligochaete populations could support between 339 and 169.6 Kg fish per ha. This represent 2.5 up to 4.5 times lower than that supplied by the benthic chironomid populations.

INTRODUCTION

This paper focuses on certain aspects regarding energy flow. The participation of the oligochaete populations to the major processes which ensure ecosystem productivity (the flow of energy and the circulation of mineral elements) depends, on one side, on their structure and, on the other side on the level and quality of the food source, on the complexity of the benthic association and, especially, on the level of the conditions of hypoxia. In order to evaluate the role played by the dominant oligochaete populations in accomplishing the productivity of the ecosystems taken into study, we determined the values of the energy budget parameters in case of each population, using on this purpose their structural data (6) and the average values of the physical and chemical parameters which conditioned this process.

1. MATERIAL AND METHOD

The general background of the analysis and interpretation of the recorded reactions in case of limnicol oligochaete associations and its components was represented in our previous paper.

The energy budget was assessed according to the generally accepted formula: $C = P + R + F + U$ and $A = P + R$ were: A = assimilated energy; C = consumption; P = production; R = respiration; F = contained energy in egesta; U = excreta.

The production was obtained using the method developed by Hamilton (1969). The production calculus is based on the distribution of the

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average value of frequency considered as ar average value of the entire population and it was obtained according to the formula :

$$P = i(\bar{x}_j - \bar{x}_{j+1}) \frac{(w_j + w_{j+1})}{2}$$ where : i = number of length class;

x_j = the average number of individuals in each length class; w_j = average weight of specimens within length class j.

The total biomass is based on the distribution of length frequency and the equation : dried weight-length.

The respiration rates were determined using a Warburg device. The experiments were achieved at 17°C and 20°C.

In order to express the dependency of respiration rate on temperature and on the age structure of the population, the multiple regression model was accepted as follows :
$\ln R = a + B_1 T + B_2 \ln w$ in which : R = respiration rate, T = temperature, w = individual dried-weight, B_1, B_2 = constants.

The avergae value of the oxicaloric coefficient 3.38 cal/mg oxygen was used in the conversion of the respiration rate into energy units (9, 10, 13).

The production (P) and respiration rate (R) being known, it was possible to evaluate the annual consumed energy (C), using the formula indicated by Winberg (1956, 1970) : $C = A \frac{1}{U^{-1}}$ or replacing A : $C = \frac{P+R}{U^{-1}}$ were U^{-1} represents assimilation efficiency which equals 0.38 for detritivorous organisms (8, 11, 14). The efficiency of consumed food utilized for growth and reproduction ($K_1 = P/C$) and the efficiency of utilization of assimilated food ($K_2 = P/A$) was obtained according to Winberg (1971). The ratio P/B was also determined.

The transit rate of the sediments through the worms was determined using an Alsterberg device (1925) modified by Brinkhurst and Appleby (1970) (1).

1. RESULTS AND DISCUSSION

2.1. Production (P), average biomass (B), and turnover rate (P/B). Knowing the populations structure and the number of generations (6), the production accomplished by each dominant population was obtained, per surface unit, during a year.

The quantity of accumulated and transferred organic matter to the next trophic level was transformed into energy units, using the caloric equivalent of the dried biomass characteristic for each population (table 1).

The populations belonging to the five species of oligochaete have the caloric equivalent of the biomass within a very restricted range (4.55—4.9 kcal/g dried biomass). These values are similar to those reported by other authors for oligochaete, within a general range of 4.5 — 5.0 kcal/g dried biomass (4, 12).

The production expressed by Kcal/sqm/year (table 2) and by Kg wet matter/ha/year (table 4), took values within large ranges, being time variable for the same population and took different values in case

Table 1

Value of energy content of the biomass accumulated by the dominant oligochaete populations

Energy equivalent	*Tubifex tubifex*	*Limnodrilus hoffmeisteri*	*B. sowerbyi*	*Ilyodrilus templetoni*	*Potamothrix hamm.*
kcal/g *	4.75	4.9	4.67	4.55	4.8

* represents dried weight

Table 2

Annual energy flow through the dominant oligochaete populations in some lakes of the Danube Delta

SPECIES	LAKE	YEAR	B	C	P	R	A	FU
P. hammoniensis	ROȘU	1976	0.057	3.848	0.684	0.583	1.27	2.578
		1977	0.022	1.482	0.264	0.225	0.489	0.993
	PORCU	1976	0.173	11.648	2.076	1.768	3.844	7.804
		1977	0.078	5 251	0.936	0.797	1.733	3.518
Br. sowerbyi	ROȘU	1976	0.027	2.023	0.34	0.368	0.708	1.315
		1977	—	—	—	—	—	—
	PUIU	1977	0.667	50.06	8.41	9.11	17.52	32.54
		1978	0.627	47.022	7.9	0.558	16.458	30.56
I. templetoni	MATIȚA	1980	0.173	19.092	3.7	3.555	7.255	11.837
		1981	0.136	9.62	1.86	1.79	3.65	5.955
		1982	0.07	5.055	0.98	0.941	1.921	3.134
T. tubifex	PUIU	1977	0.048	4.422	1.026	0.743	1.769	2.654
		1978	0.026	2.397	0.556	0.403	0.959	1.438
	MERHEI	1980	0.058	5.345	1.24	0.898	2.138	3.207
		1981	0.034	3.132	0.727.	0.526	1.253	1.879
		1982	0.028	2.577	0.598	0.433	1.031	1.546
	MATIȚA	1980	0.082	7.555	1.753	1.269	3.022	4.533
		1981	0.058	5.345	1.24	0.898	2.138	3.207
		1982	0.072	6.632	1.539	1.114	2.653	3.979
L. hoffmeisteri	PUIU	1977	0.24	25.66	6.468	4.312	10.78	14.886
		1978	0.194	20.745	5.228	3.485	8.713	12.03
	ROȘU	1976	0.038	4.064	1.024	0.683	1.707	2.357
		1077	0.016	1.7	0.43	0.287	0.714	0.986
	MERHEI	1980	0.033	3.258	0.889	0.593	1.482	2.046
		1981	0.025	2.674	0.674	0.449	1.123	1.55
		1982	0.02	2.138	0.539	0.359	0.898	1.24

R = average biomass (dried weight); C = energy input; P = stored energy (production); B = cost of maintenance; A = assimilated energy; FU = not used energy. (Values for C,P, R,A,FU are expressed in kcal/sqm/year).

of populations belonging to the same species, but integrated into different ecosystems and also differentiated relative to species.

It can be emphasized the high level of production (7.9 — 8.4 Kcal/sqm/year) achieved by *Branchiura sowerbyi* in lake Puiu.

For the same lake a very important role as energy carrier has had the *Limnodrilus hoffmeisteri* population, which stored and reintroduced into the energy flow an amount of 5.228 — 6.468 Kcal/sqm/year.

Large amounts of energy and organic matter have been taken out from the sediments and reintroduced into the energy flow by the *Ilyodrilus templetoni* population in lake Matita just in 1980 (3.7 Kcal/sqm/year), by the *Potamotrix hammoniensis* population (in 1976) in lake Porcu (2.076 Kcal/sqm/year) and by the *Tubifex tubifex* population in lake Matita (during the whole research period): 1.24 — 1.75 Kcal/sqm/year.

The *T. tubifex* populations in lakes Puiu and Merhei, the *L. hoffmeisteri* populations in lakes Rosu and Merhei, as well as the *B. sowerbyi* and *P. hammoniensis* populations in lake Rosu have contributed at a lesser extent to the process of recycling of the energy stored in the sediments (usually P $<$ 1 Kcal/sqm/year).

The average biomass (B) was over 0.6 Kcal /sqm/year and 55 Kg/ha/year respectively only in case of *B. sowerbyi* in Puiu lake. For the other populations the biomass (B) was lower (tables 2, 4).

Biomass turnover rate characterized by the P/B ratio has fluctuated in close connection with the number of generations achieved by the dominant populations belonging to the five oligochaete species. The *B. sowerbyi*, *P. hammoniensis* and *I. templetoni* populations (in 1981 and 1982), which had only one generation per year, have recycled 2.7, 2.5 and respectively 3 times the average biomass during a year. The *T. tubifex* populations and the *I. templetoni* ones (in 1980), which have produced two generation per year, have recycled 4.5 and respectively 4.7 times the average biomass, while the *L. hoffmeisteri* populations, with 2 — 3 generations per year, have recycled 5.5 times the average biomass respectively, the energy storage (table 3).

Table 3

Values of the P/B, U^{-1}, K_1, K_2 coefficients for the dominant oligochaete populations

Coefficient	*Tubifex tubifex*	*Limnodrilus hoffmeisteri*	*Branchiura sowerbyi*	*Ilyodrilus templetoni*	*Potamothrix hammoniensis*
P/B	4.5	5.5	2.7	4.7(3)*	2.5
$U^{-1} = A/C$	0.4	0.42	0.35	0.38	0.33
$K_2 = P/A$	0.5	0.6	0.48	0.51	0.54
$K_1 = P/C$	0.23	0.252	0.168	0.194	0.178

* the value in parentheses corresponds to the interval when the population has produced one single generation per a year.

2.2. Cost of maintenance (R) and efficiency of utilization of assimilated energy (K2).

The data regarding the oxygen consuming rate show that there are no differences among the populations of the *T. tubifex*, *L. hoffmeisteri*, *P. hammoniensis* and *I. templetoni* species, a fact which determined us to deal with the data in a unitary way and to differentiate a single model of simple regression in order to describe oxygen consumption in dependence of the weight. The correlation of these parameters is described by the equations: $R = 1.32\ W^{0.56}$ for *B. sowerbyi* and $R = 0.57\ W^{0.63}$ for other populations, were R = μl O_2/mg and W = wet biomass.

The value of the correlation coefficient (0.91 for *B. sowerbyi* and 0.85 for other populations) shows a high dependence of the oxygen consumption rate on the weight. The data regarding the dependency of the oxygen consumption rate on temperature, have permitted us to evaluate the value of Q_{10} coefficient that is 1.5 for *B. sowerbyi* populations and 1.85 for the other studied populations.

For different species of Tubificides, values for the Q_{10} coefficient have been reported ranging between 1.6 — 2.1 (2, 4).

In dependence on their structure and on the average monthly temperature, the values of the energy loss in life processes by the dominant oligochaete populatios was estimated within a large range: 0.225 — 9.11 Kcal/sqm/year (table 2).

The highest level of utilization for growth of assimilated energy (K_2) was recorded in the case of *T. tubifex* (58%) and *L. hoffmeisteri* (60%) populations. For the *T. tubifex* and *L. hoffmeisteri* species in experimental conditions it was established that up to 63% of the assimilated energy, can be stored and transferred to the next trophic level (4). It is possible that when tubificidae populations obtain partially the energy through the anaerobically path, the K_2 coefficient will be lower.

2.3. The ingested energy from the sediments (C), assimilation efficiency (U^{-1}) and coefficient of consumed food utilized for growth (K_1).

By using the assimilation efficiency of the five oligochaete species (table 3) and their production and respiration, it was possible to calculate for each population the energy ingested from the sediments (C) during a year (table 2). It can be noticed that *P. hammoniensis* populations have intake between 1.482 (Rosu, 1977) — 11.648 Kcal/sqm/year (Porcu, 1976), those of *B. sowerbyi* species from 2.02 (Rosu, 1976) up to 19.09 Kcal/sqm/year (Matita). The *T. tubifex* populations consumed between 2.397 (Puiu, 1978) and 7.55 Kcal/sqm/year (Matita, 1980), while those of *L. hoffmeisteri* species between 1.7 (Rosu, 1977) and 25.66 Kcal/sqm/year (Puiu, 1977).

Regarding the efficiency level of consumed food utilized for growth (K_1), the *B. sowerbyi* populations stored only 16.8%, while those of *L. hoffmeisteri* stored 25.2% of the consumed energy (table 3). For the dominant populations belonging to the other oligochaete species, intermediate values were recorded.

2.4. Energy transferred by oligochaete populations from the sediments (B1), to the benthivorus fishes, fish biomass estimation (B_2) and their production (P_2).

In the diagrams representing the structural model of the Rosu, Puiu, Porcu and Matita-Merhei aquatic ecosystems, the benthic fauna was diffe-

rentiated as a key compartment integrated into one of the main channels of energy (3. 15).

The trophic basis supplied by the oligochaetes for the benthivorus fishes was achieved in different proportions by the populations of the five species.

In Puiu lake the trophic basis was achieved 70 — 74% by *B. sowerbyi* population and 23 — 25% by *L. hoffmeisteri* ones. In Matita lake the trophic basis was achieved 49 — 70% and 30 — 51%, respectively, by the *I. templetoni* and *T. tubifex* populations, while in Merhei lake 58 — 64% and 36 — 42% respectively, by the populations belonging to the *T. tubifex* and *L. hoffmeisteri* species. In Rosu lake the trophic basis represented by the oligochaetes was achieved 47 — 58% and 31 — 42% by *P.*

Table 1

Estimated values of the benthivorus fishes biomass (B_2) and of their production (P_2) depending on the biomass (B_1) and production (P_1) of the dominant oligochaete populations

LAKE	YEAR	B_1*	B_1 SUPPLIED BY :	P_1*	B_2*	P_2*	P_2*[1]
ROȘU	1976	8.54	47% *P. hammoniensis* 31% *L. hoffmeisteri* 22% *Br. sowerbyi*	29.7	45.46	4.3	37.8
	1977	2.26	58% *P. hammoniensis* 42% *L. hoffmeisteri*	10.6	33.9	1.4	
PUIU	1977	66.85	70% *Br. sowerbyi* 25% *L. hoffmeisteri* 5% *T. tubifex*	233.6	169.6	33.6	23.6
	1978	59.3	74% *Br. sowerbyi* 23% *L. hoffmeisteri* 3% *T. tubifex*	201.4	153.7	29.0	
PORCU	1976	12.1	100% *P. hammoniensis*	30.3	74.6	4.3	81.4
	1977	5.46	100% *P. hammoniensis*	13.6	49.4	2.0	
MATIȚA	1980	17.85	68% *I. templetoni* 32% *T. tubifex*	82.7	69.3	11.9	1.9
	1981	13.58	70% *I. templetoni* 30% *T. tubifex*	46.8	59.6	6.7	5.1
	1982	9.94	49% *I. templetoni* 51% *T. tubifex*	37.4	51.3	5.4	22.5
MERHEI	1980	6.37	64% *T. tubifex* 36% *L. hoffmeisteri*	31.0	52.9	4.5	4.3
	1981	4.1	58% *T. tubifex* 42% *L. hoffmeisteri*	20.3	44.4	2.9	3.6
	1982	3.36	58% *T. tubifex* 42% *L. hoffmeisteri*	16.5	41.4	2.4	11.9

* represent wet weight (kg/ha/year)

[1] represent expected fish production supported by the energy stored by the dominant chironomide populations (over 90% by *Chironomus plumosus*) (8)

hammoniensis and *L. hoffmeisteri* populations respectively, while in Porcu lake exclusively by *P. hammoniensis* populations.

The results (table 4) indicate that during the research period the trophic basis represented by the dominant oligochaete populations could support between 33.9 and 169.6 Kg fish/ha.

In Puiu lake, the fish production supported by the oligochaete biomass was 2 — 4 times higher than in other lakes.

The production that could be achieved by the benthivorus fishes (P_2) by using the trophic basis supplied by the oligochaete populations was estimated using the following actual criteria :

a) 90% of the energy stored by oligochaete populations was transferred to benthivorus fishes, 10% being used for oligochaete reproduction (17) ;

b) the fish assimilation efficiency (U^{-1}) was 80% ;

c) the efficiency of the utilization for growth of assimilated energy (K_2) by the benthivorus fishes was 20% (17).

The same criteria were used to estimate the benthivorus fish productive potential depending on the trophic basis achieved by the dominant populations of chironomids of the Danube Delta aquatic ecosystems (8).

The energy transferred by the oligochaete populations (prevalent by *B. sowerbyi*) in Puiu lake can support a high level of productivity of the benthivorus ihtiophauna (29 — 33.6 Kg/ha/year). For the other lakes a low level of fish production was observed. In the case of Matita and Merhei lakes, on the background of the considerable decrease in the energy carrier function of the oligochaete populations, caused by the rapid development towards a hypertrophic state, a corresponding diminishing of the benthivorus fishes productive potential is obvious.

Comparing the benthivorus fishes productive potential supported by the trophic basis represented by the dominant oligochaete populations with those supported by the chironomides ones (3, 8) the following can be observed :

— the production of the benthivorus fishes supported by the oligochaete populations represented only 3.7 — 7.0% in Porcu and Rosu lakes and about 57% in Puiu lake from the fish production supported by the entire benthic fauna (dominated by chironomids and oligochaetes) :

— in Matita and Merhei lakes as the hypertrophic state was accentuated the fish production supported by oligochaete populations was reduced from 85% to 19% and from 50% to 17% respectively, during the 1980 — — 1982 interval.

Based on the elements given in this analysis, we state that in the Danube Delta aquatic ecosystems which are in an advanced phase of eutrophication, the benthic oligochaete supply a trophic basis for the benthic ihtiofauna 2.5 — 4.5 times lower than that supplied by the benthic chironomid populations (especially that of the *Chironomus plumosus*).

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DAS BAKTERIEN—PLANKTON IN DER DONAU IM GEBIET DES EINTRITS INS DELTA IM ZEITRAUM 1991—1992

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This paper presents the bacterioplankton in Danube from the entrance of the river in Romania to Danube Delta. Dinamics and evolution of bacterioplankton are analysed during the period 1981 — 1992 with numerical density and biomass details for 1991 — 1992. There is also a synthesis of our investigations and of those from the literature concerning bacterioplankton of the lower section of Danube during 1975 — 1992.

Vorliegende Arbeit bringt Daten über den Bakterienplankton der Donau im Gebiet des Eintritts ins Delta : Donau-Stromkm. 82 im Ceatal Izmail und Donau —Stromkm. 63 im Ceatal Sf. Gheorghe sowie auch in anderen Zonen des rumänischen Abschnittes (km 1076).

Das Bakterienplankton der Donau wird aus zwei Gesichtspunkten untersucht : 1) — die Dynamik der nummerischen Dichte während der letzten zwei Jahre (1991 — 1992) sowie auch die Evolution des Bakterienplanktons während einer längeren Zeitspanne (1981 — 1992) (2) und 2) — die Veränderungen der Biomasse während der Periode (1981 — 1992) (6, 7). Die Ergebnisse betreffend den Bakterienplankton des unteren Abschnittes der Donau während der Jahre 1975 — 1992 werden in Zusammenhang mit den Daten aus der Fachliteratur dargestellt. In diesem Kontext beziehen sich unsere Beobachtungen auf folgende Abschnitte : Km

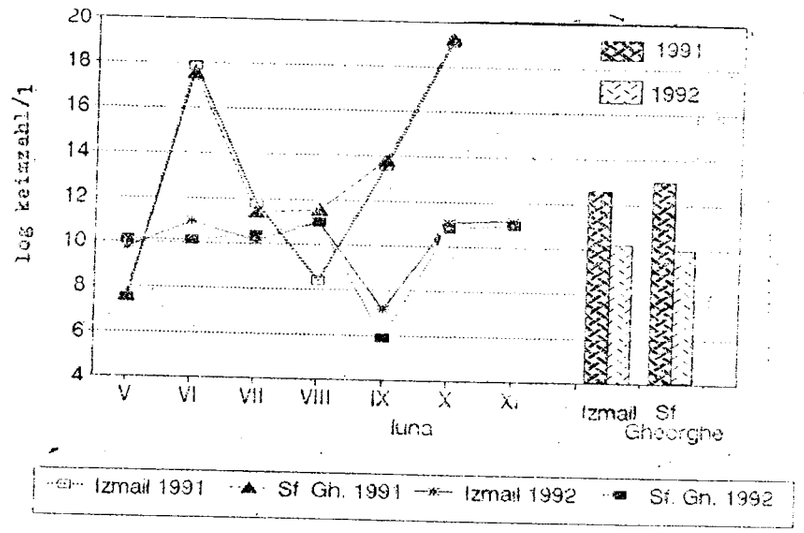

Abb. 1. — Die Zahlenmässige Dichten dinamik aus Bakterien-Plankton im 1991 — 1992 Jahren

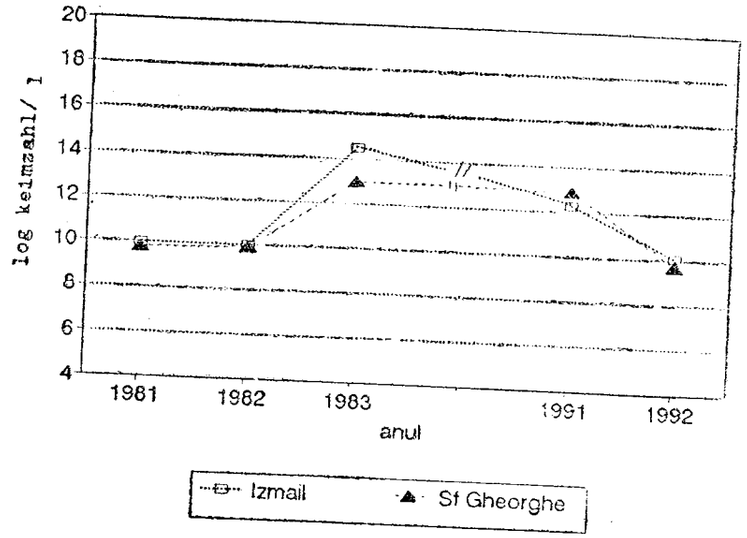

Abb. 2. — Die Bakterien-Plankton Evolution aus der Donau bei ihren einmundung ons Delta.

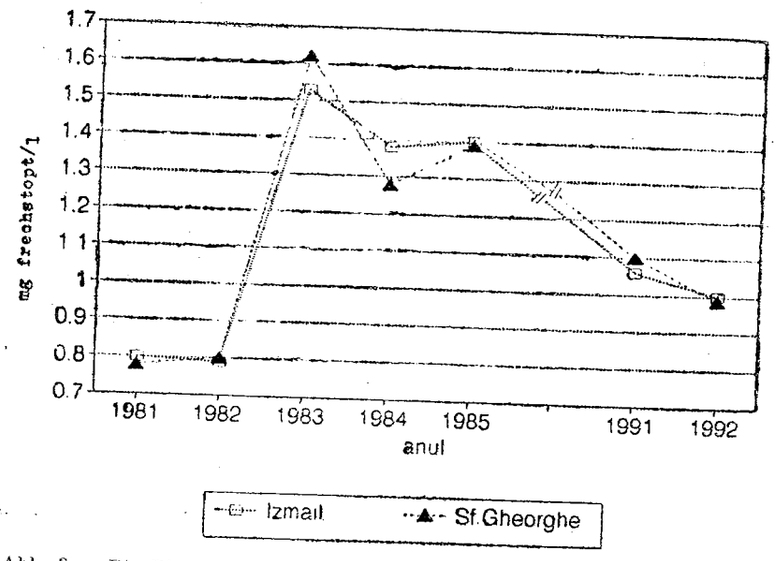

Abb. 3. — Die Biomasse des bakterienplanktons aus Donau bei ihren einmundung ins Delta.

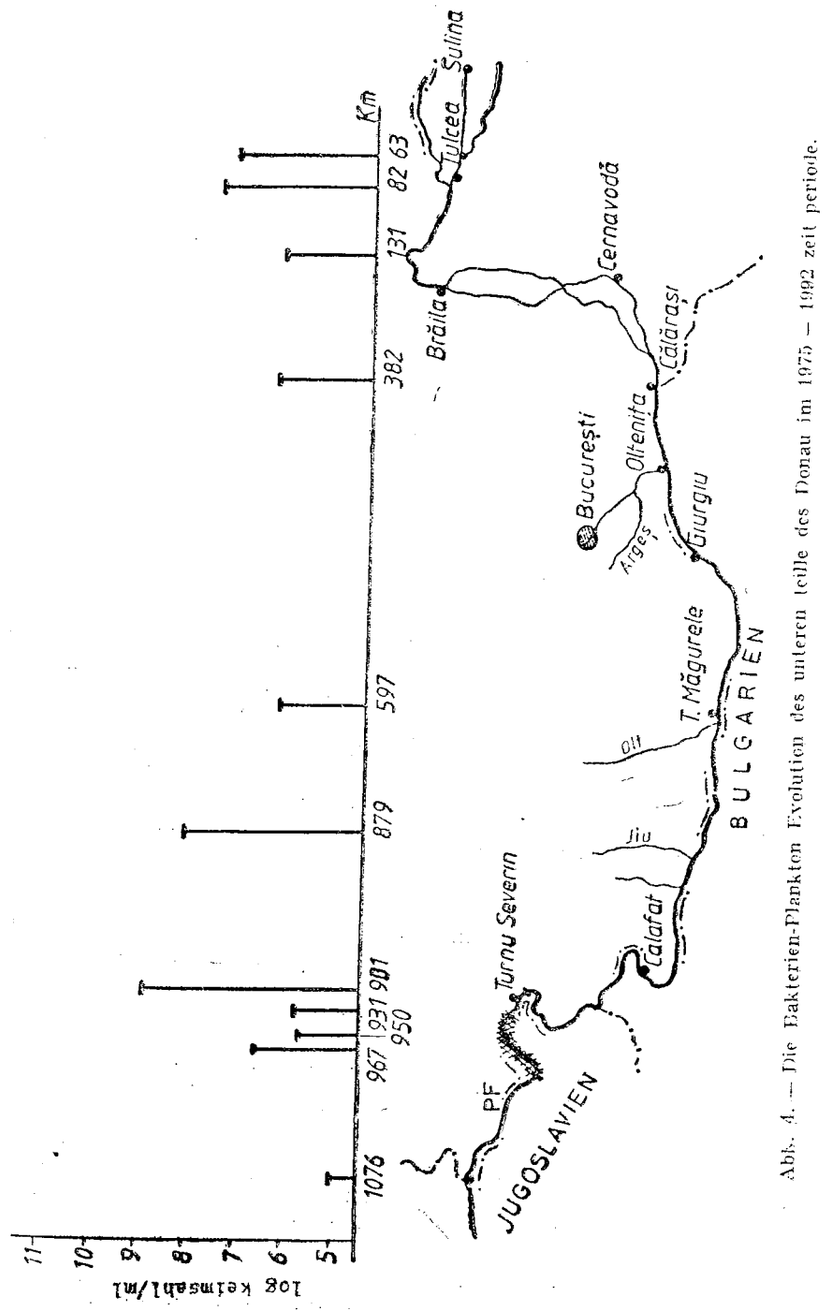

Abb. 4. — Die Bakterien-Plankton Evolution des unteren Teille des Donau im 1975 — 1992 zeit periole.

1076 (unaufgelegene Daten), Km 967 ; 950 (1, 4) ; Km 931 ; 901 ; 878 (5) ; Km 597 ; 382 ; 131 (3), Km 92 und 63 (2).

Die monatliche Dynamik der nummerischen Dichte der Gesamtzahl der heterotrophen Keime (welche auf Gelose-Nährböden bei 20° 48 h bestimmt wurden) wiederspiegelt den Einfluß der hydrologischen Faktoren während der erwähnten Zeitspanne 1991—1992. (Abb 1). Im jahre 1991 werden die Maxima in den Monaten Juni und Oktober vermerkt, während die Minima in den hervorgehenden Monaten Mai und September (wann die Frühlings — und Herbst — Anschwemmungen stattfinden) kennzeichnet werden. Eine ähnliche Situation ist auch für das Jahr 1992 kennzeichnend mit dem Unterschied daß die monatlichen Werte mit 3—4 Ordnungsgrößen kleiner sind im Vergleich mit dem vorrigen Jahr. Diese Tatsache ersieht man aus den jährlichen Mittelwerten und aus den Schwankungsgrenzen der nummerischen Dichte und zwar : $10^8 - 10^{16}$ im Jahre 1991 und 10^2—10^8 im Jahre 1992. Beziehen wir uns auf die pZeitspanne 1981 — 1983, so können wir die Daten aus dem Jahre 1983 betreffend die jährlichen Mittelwerte der nummerischen Dichte mit denen aus dem Jahre 1992 vergleichen, während im Jahre 1992 eine ähnliche Situation mit den Jahren 1981 — 1982 kennzeichnet wird (Abb 2).

Die Biomasse (die jährlichen Mittelwerte wurden aufgrund der Regressionsgleichung $\log Y = -0{,}801579 - 0{,}930129 \log x$ berechnet, in welcher x = Anzahl der Keime/l und Y = Biomasse der Keime darstellen) zeigt einen wertmässigen Abstieg der mikrobiellen Belastung im Vergleich mit dem Jahr 1984 — 1985 aber man kommt nicht zu der Situation des Jahres 1981 — 1982 (Abb. 3).

Die Qualitat der Donau vom Eintritt in unserem Land bis zur Mundung ins Meer wiederspiegelt sich durch die Dynamik der mikrobiellen Belastung ganz besonders in stark industralisierten Zonen oder in Agrarzonen. (Abb 4).

Die chemische Wasserverschmutzung welche durch Industrie-, Agrikultur- und Städte-Abfließenlassen entstammt beeinträchtigt die Donau in verschiedenen Abschnitten-mit verschiedener Intensität.

Wegen der großen Durchflußmenge der Donau und der erheblichen Selbstreinigungsfähigkeit des Wassers werden die Verschmutzungseffekte des Wassers auf den großten Teil der Donau vermindert ; doch werdden in dieser Zone große Mengen von Suspensionen angehäuft welche die Entwicklung des Bakterienplanktons in dieser Zone auf das 3 — 4 fache vergrößern in gegenüber des Eintritts in des rumänischen zone.

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