COMITÉ DE RÉDACTION

Rédacteur en chef:

EUGEN A. PORA, membre de l'Académie de la République Socialiste de Roumanie

Rédacteur en chef adjoint:

R. CODREANU, membre correspondant de l'Académie de la République Socialiste de Roumanie

Membres:

MIHAI A. IONESCU, MIHAI BĂCESCU, OLGA NECRASOV, GRIGORE ELIESCU, membres correspondants de l'Académie de la République Socialiste de Roumanie; MARIA CALOIANU, secrétaire de rédaction.

Toute commande de l'étranger (fascicules ou abonnements) sera adressée à ROMPRESFILATELIA, Boîte postale 2001 -- telex 011631 — Bucarest, Roumanie ou à ses représentants à l'étranger.

Les manuscrits, les livres et les revues proposés en échange, ainsi que toute correspondance, seront envoyés à la rédaction: 296, Splaiul Independenţei, Bucarest, Roumanie

REVUE ROUMAINE DE BIOLOGIE

— SÉRIE DE ZOOLOGIE —

TOME 17 1972 Nº 2

SOMMAIRE

Rev. roum. biol. — zoologie, tome 17, Nº 2, p. 77—140, Bucarest, 1972

FREE-LIVING MARINE NEMATODES FROM THE BLACK SEA. DESCRIPTION OF THREE NEW SPECIES

BY

ELENA GROZA-ROJANCOVSKI

Three new species of marine nematodes are described. Two of them belong to genera never known in the Black Sea, *Steineria* and *Halanonchus*.

The present study was carried out in the Nematology Laboratory of the Gent University (Belgium) — Director : Prof. Dr. L. de Coninck.

The material has been supplied through the kindness of Prof. Dr. M. Băcescu, director of the Museum of Natural History "Grigore Antipa", Bucharest. It derives from several stations (10—70 m. deep) of the Black Sea.

These stations have been achieved by Romanian researchers in the framework of the program of scientific investigation of the Romanian continental coast, over the 1954 and 1959 period [1].

The method of Dr. de Grisse [4] was used.

The three new species presented were found in the same station : *St. 456*, 30—IX—1956 ; 43°49′ N, 29°24′ E ; salinity 18.1 g‰, at 64 m. in depth, on grey silt.

ORDER OF MONHYSTERIDA

LINHOMOEIDAE

Paralinhomoeus deconincki n.sp. (Fig. 1, *A*, *B*).

Holotype : δ_1 slide 3, deposited in the collection of the Museum of Natural History "Gr. Antipa", No. 179.
Allotype : ♀ in the same slide, under the same number.
Paratype : δ_2 slide 7, in the same collection, No. 180.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, Nº 2, p. 79—85, BUCAREST, 1972

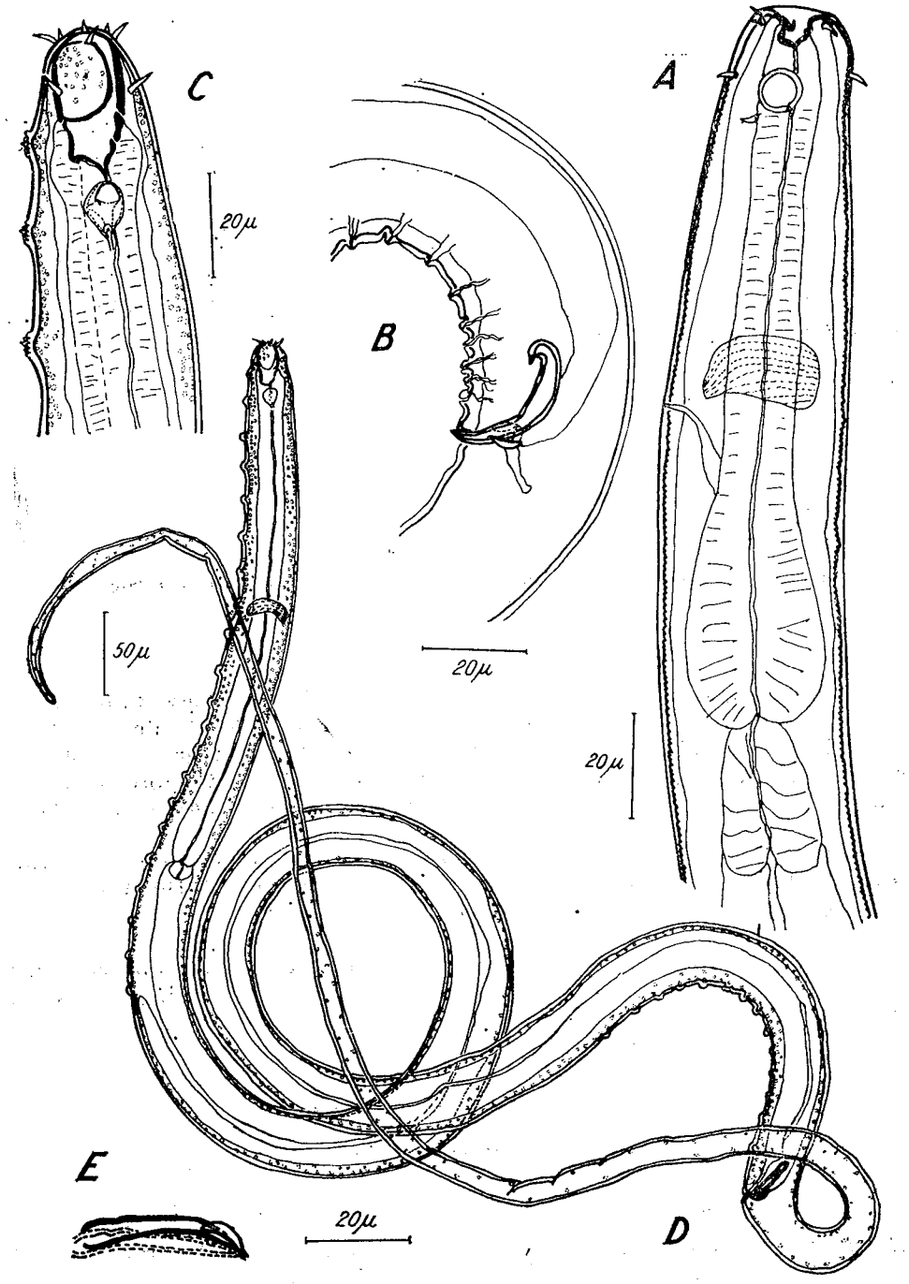


Fig. 1. — *Paralinhomoeus deconincki* n. sp. *A*, anterior end; *B*, anal region of male. *Halanonchus papilatus* n.sp. *C*, anterior end; *D*, general view; *E*, spicula.

Description :

δ_1 : L = 4 mm.

Filipjev's formula :

$$\delta_1 \frac{-\ 110\ \ 130\ \ 220\ \ M\ \ 3600}{32\ \ 55\ \ \ 57\ \ \ 57\ \ \ 57\ \ \ 50}\ 4000\ \mu\ \ a:72\ b:18\ c:10$$

$$\varphi \frac{-\ 100\ \ 130\ \ 230\ \ V\ (2000)\ \ 4050}{35\ \ 55\ \ \ 55\ \ 60\ \ \ 70\ \ \ \ \ \ \ 40}\ 4500\ \mu\ \ a:64\ \ \ b:19\ \ \ c:10\ \ V:44\,\%$$

$$\delta_2 \frac{-\ \ -\ \ 300\ \ M\ \ 4150}{31\ \ -\ \ \ 52\ \ 52\ \ 45}\ 4750\ \mu\ \ a:86\ \ \ b:15.8\ \ \ c:10.3$$

Nematodes of large size, filiform, slender, regularly attenuating toward extremities.

Cuticle smooth, finely striated.

Head blunt, anterior attenuation, 50%. Six 6 μ cephalic setae, six setae a little shorter; four 6 —7 μ subcephalic or cervical setae.

Amphid circular, cuticularized walls, situated at 22—25 μ from anterior end (0.7 —0.8 cephalic diameter); 12 —14 μ in diameter (0.28 corresponding diameter). Just before the amphid, the 4 subcephalic setae are inserted.

Buccal cavity typical, strongly cuticularized, 8 μ deep, 6 μ wide.

Esophagus slightly swollen behind the buccal cavity, then cylindric, forming at the posterior extremity a 65 —75 μ long widening (1.4 corresp. diameter).

Cardia well developed, elongated, 55 μ long; 1.5 time wider toward intestinal portion than toward the esophageal one.

Nerve-ring situated at 0.50 esophageal length.

Excretory pore behind the nerve-ring, sometimes difficult to observe.

In males, *spicula*, 40 μ long, 0.88 anal diameter, are curved, their proximal extremity slightly widened and opened on the ventral side.

Gubernaculum presents a caudal apophysis 12 —15 μ long, a more or less rectangular median body, bearing two lateral grooves.

Before the anus, a series of 10 well-developed preanal papillae.

Tail 400 —600 μ long, or 10 anal diameters, is firstly conical (1/4 of its length), then filiform.

Females have two right *ovaries*, the anterior ovary more developed than the other.

Discussion. Paralinhomoeus deconincki n.sp. is clearly related to *Paralinhomoeus caxinus* Vitiello, 1969 [5] by the distribution and length of the cephalic setae, yet the subcephalic setae are longer, the amphid is situated more posteriorly and the size is larger.

This species also differs from all the other species of the genus by the presence, in males, of a series of preanal, well-developed papillae.

MONHYSTERIDAE

Steineria pontica n.sp. (Fig. 2 *A*, *B*, *C*).

Holotype : δ_1 slide 12, deposited in the collection of the Museum of Natural History "Gr. Antipa", No. 181.

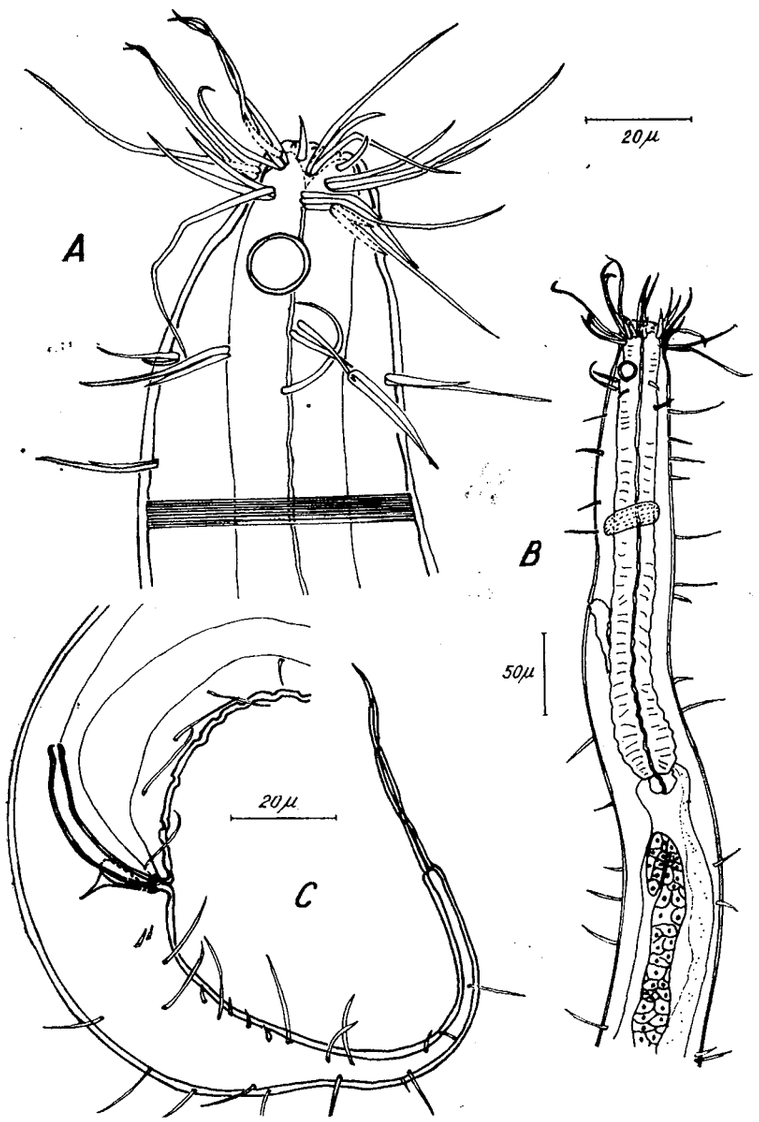


Fig. 2. — *Steineria pontica* n. sp. *A*, anterior end of male; *B*, anterior end of female; *C*, tail of male.

Paratype : δ_2 in the same slide, under the same number.
Allotype : ♀ slide 2, in the same collection, No. 182.
Description :

δ_1 : L = 1.3 mm.
Filipjev's formula :

$$\delta_1 \frac{-\ 110\ -\ 220\ \text{M}\ 1{,}100}{25\ \ 50\ -\ \ 55\ \ 55\ \ 45} 1{,}300\ \mu\ \text{a} : 23.6\quad \text{b} : 5.9\quad \text{c} : 6.5$$

$$\delta_2 \frac{-\ 100\ -\ 220\ \text{M}\ 1{,}125}{25\ \ 53\ -\ \ 59\ \ 59\ \ 48} 1{,}300\ \mu\ \text{a} : 22\quad \text{b} : 6\quad \text{c} : 7$$

$$♀ \frac{-\ 120\ \ 180\ \ 260\ \text{V}\ 1{,}240}{25\ \ 50\ \ 55\ \ 60\ \ 60\ \ 45} 1{,}450\ \mu\ \text{a} : 21.8\quad \text{b} : 6.3\quad \text{c} : 6.7$$

$$\text{juv.} \frac{-\ 110\ \ 170\ \ 260\ \text{M}\ 1{,}320}{25\ \ 50\ \ 55\ \ 60\ \ 60\ \ 47} 1{,}520\ \mu\ \ \text{a} : 25\quad \text{b} : 5.8\quad \text{c} : 7.6$$

Body slightly attenuated anteriorly (anterior attenuation : 45%), tapering towards posterior end.

Cuticle with fine striations ; somatic setae distributed on the body, more numerous on the tail.

Head circular ; 6 distinct lips and 6 small papillae on each lip.

The cephalic setae arranged in two crowns : anterior crown : 6 setae, 7 μ long, and a posterior crown : 24 setae by groups of 3 each, 35—38 μ long. A little behind this level, 8 groups of 3 subcephalic setae, 30—35 μ long.

Amphid circular, 10.5 μ in diameter (28% of the corresp. diameter), situated at about 25 μ from the anterior end (1 cephalic diameter, or less).

Buccal cavity typical, trumpet-shaped, yet large enough.

Esophagus muscular, mostly cylindrical, widened to the base.

Cardia very small.

Nerve-ring, sometimes difficult to observe, situated at 0.5 esophageal length ; *excretory pore*, at 0.65 of the same length.

Spicula of 55 μ (cord) : 1.2 anal diameter, slightly curved.

Gubernaculum of small size, well delimited, provided with posterior apophysis, 22 μ long.

In males, before the anus, 8 well-developed papillae and numerous setae.

Ovary simple, 640 μ long. Vulve situated at 56% from the anterior end, muscular enough.

Tail, 200 μ long (4.4 anal diameters), conical in shape. Its extremities are provided with two setae, 17 μ long.

Discussion. Tnis species resembles *Steineria longicaudata* Vitiello, 1970 [6] ; differs from it by the arrangement of the cephalic setae, position of amphid, more anterior in our species, shape and size of spicula and gubernaculum.

An important character is the presence of preanal papillae, not found in other species described in literature.

The genus *Steineria* is described for the first time in the Black Sea.

ORDER OF ARAEOLAIMIDA

HALANONCHINAE

Halanonchus papilatus n.sp. (Fig. 1, *C*, *D*, *E*).

Holotype : δ_1 slide 8, deposited in the collection of the Museum of Natural History "Gr. Antipa", No. 183.
Paratype : δ_2 slide 10, in the same collection, No. 184.
Description :

δ_1 : L = 2.6 mm.
Filipjev's formula :

$$\delta_1 \frac{- - - 330\ M\ 1{,}500}{15 - - 35\ 35\ 30} 2{,}600\ \mu \quad a : 74 \quad b : 7.8 \quad c : 2.3$$

$$\delta_2 \frac{- - 140\ 350\ M\ 1{,}700}{15 - 30\ 35\ 35\ 30} 2{,}800\ \mu \quad a : 80 \quad b : 8 \quad c : 2.5$$

Body elongated, slightly attenuated anteriorly, very tapered toward posterior end.

Cuticle smooth, but over the cuticle, in the epiderm, a high amount of refractory granules.

Anterior end, more or less rounded. Labial papillae non visible ; 12 cephalic setae : an anterior crown — 6 setae, 3 μ each — and a posterior crown — 6 longer setae, 6 μ. Four subcephalic setae, 7 μ long.

Amphid situated 20 —21 μ from anterior end (1.1 cephalic diameter), fairly pocket-shaped, but inside, a more or less convoluted structure is observed. Opening, almost circular, 8 μ in diameter.

Buccal cavity, large, cylindrical, 20 μ deep, 11 μ wide, walls thickly cuticularized.

In males, a series of ventral papillae starting from the last third, continues along the esophagus. Each papilla has a basin-like aspect, with doubly toothed edges and a central papilla.

Papilla has 4 μ in diameter and the interval between two papillae, 10 —12 μ.

Esophagus cylindric, almost uniform, widening toward the base. Small *cardia*, 10 μ long.

Nerve-ring at 0.40 esophageal length, difficult to see on account of refractory granules.

Spicula short : 35 μ (cord), 1.1 anal diameter, thin, slightly curved.

Gubernaculum a little less than one-fourth of spicula length, linear, or slightly curved ; 14 preanal papillae, of the same structure as the cervical ones.

Tail very long : 1,100 μ (36.6 anal diameters), tapered.

Discussion. Halanonchus papilatus n.sp.related to *Halanonchus macrurus* Cobb, 1920 [2]. It differs from this species by the shape and structure of amphids, the presence of 21 structured cervical papillae, which are hardly noticed in *H. macrurus* and only in number of 5 —6 : Wieser and Hopper, 1967 [7].

The gubernaculum of our species, well delimited, with a certain thickness and the preanal papillae represent a differentiating character.

The genus *Halanonchus* is rarely found in European waters. In the Black Sea it is noted for the first time.

REFERENCES

1. Băcescu M., Müller G. I., Gomoiu M. T., *Ecologie marină*. Ed. Acad., Bucharest, 1971, **4**, 146—155.
2. Cobb N. A., *One hundred new nemas*. Contr. Sci. Nematology, Baltimore, 1920, **9**, 217—343.
3. Gerlach S. A., Zool. Jb. (Syst.), 1963, **90**, 594—658.
4. Grisse A. T. de, Meded. Rijkfak. Landbouw. Weten. (Gent), 1969, **34**, *2*, 351—369.
5. Vitiello P., Téthys, 1969, **1**, *2*, 493—526.
6. — Téthys, 1970, **2**, *3*, 647—690.
7. Wieser W., Hopper B. E., Bull. Mus. Comp. Zool. Harv., 1967, **135**, *5*, 239—344.

Received November 24, 1971

The "Grigore Antipa" Museum of Natural History

ICHNEUMONIDES NOUVEAUX (HYM. ICHNEUM.) DANS LA FAUNE DE LA ROUMANIE

PAR

MIHAI I. CONSTANTINEANU et GHEORGHE MUSTAȚĂ

In this paper, the authors mention :
I. Two species new for science : 1. *Nepiera molaavica*, ♀♂ and 2. ***Hemi**leles moldavicus*, ♀.
II. Six species new for the fauna of Romania are also recorded as follows : 1. *Scambus eucosmidarum* Perk., ♀, 2. *Homotropus asyntactus* Schm., ♂, 3. *Diadegma gibbula* Brisch, ♀♂, 4. *Phygadeuon trichops* Thoms., ♀♂, 5. *Ph. geniculatus* Kriechb., ♂, and 6. *Stilpnus gagates* Grav., ♀♂.

Dans le présent travail les auteurs signalent deux espèces nouvelles pour la science et six espèces nouvelles pour la faune de la République Socialiste de Roumanie. Toutes ces espèces ont été obtenues par des cultures d'insectes nuisibles aux cultures potagères. Nous donnons une carte (fig. 1), avec les localités dans lesquelles ont été faites les observations. Nous avons consulté la bibliographie suivante [1—8].

Famille des **ICHNEUMONIDAE** Haliday, 1838.

A. Section PIMPLOIDAE Dalla Torre, 1901.

Sous-familles des ***EPHIALTINAE*** Townes et collab., 1960.

Tribu des **SCAMBINI** Constantineanu et Ciochia, 1967.

I. Le genre **Scambus** Hartig, 1838.

1. **Scambus eucosmidarum** Perkins, 1957, ♀.

1 ♀, éclose le 7.X.1969 d'une chrysalide de *Depressaria nervosa* Haw., récoltée à Roman, le 25.IX.1969.

Ecologie : inconnue antérieurement.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, Nº 2, p. 87—95, BUCAREST, 1972

Répartition géographique : Europe.

Nous mentionnons l'espèce *Depressaria nervosa* Haw. pour la première fois dans la science comme hôte pour cette espèce.

Espèce nouvelle pour la faune de la Roumanie.

B. Section TRYPHONOIDAE Dalla Torre, 1901.

Sous-famille des ***DIPLAZONTINAE*** Hopper, 1959.

II. Le genre **Homotropus** Förster, 1868.

2. **Homotropus asyntactus** Schmiedeknecht, 1927, ♂.

1 ♂, éclose le 7.VI.1969 d'une pupe de *Chortophila brassicae* Bouché, récoltée à l'état de larve, le 26.V.1969 dans la commune de Homocea, département de Vrancea. La transformation en pupe s'est produite le 29.V.1969.

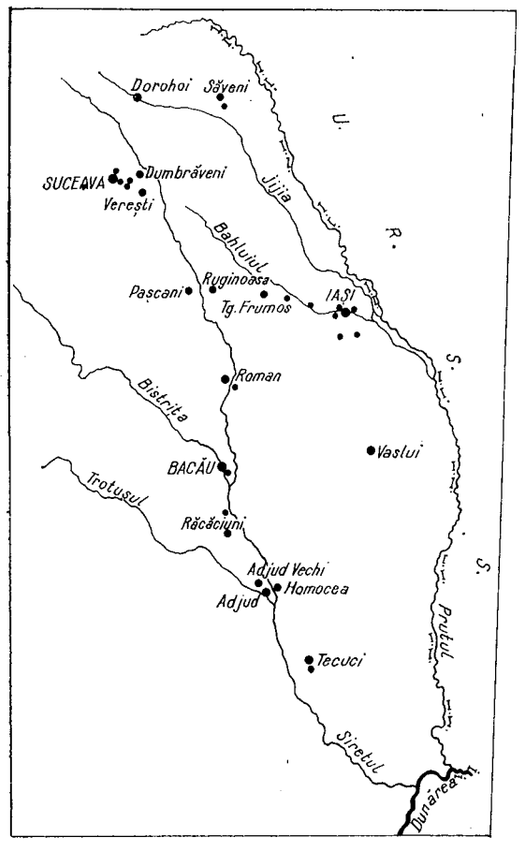


Fig. 1. — La carte de la Moldavie, avec les localités dans lesquelles ont été faites des observations.

Ecologie : inconnue antérieurement.

Répartition géographique : République Démocratique Allemande.

Nous signalons l'espèce *Chortophila brassicae* Bouché pour la première fois dans la science comme hôte pour cette espèce.

Espèce nouvelle pour la faune de la Roumanie.

C. Section des OPHIONOIDAE Dalla Torre, 1901.

Sous-famille des ***CAMPOPLEGINAE*** Dalla Torre, 1901.

III. Le genre **Nepiera** Förster, 1868.

3. **Nepiera moldavica** nov. sp., ♀♂.

L'holotype ♀ et l'allotype ♂ ont été éclos de chrysalides de *Plutella maculipennis* Curt., le 29.VII.1970 (♀) et le 26.VII.1970 (♂), récoltés à Răcăciuni, département de Bacău, le 23.VII

1970. Le paratype ♀, éclos le 14.VIII.1970 d'une chrysalide de *Plutella maculipennis* Curt., récoltée le 4.VIII.1970, à Săveni, département de Botoşani, leg. Gh. Mustață.

L'holotype, l'allotype et le paratype se trouvent dans la collection Mihai I. Constantineanu.

♀. Longueur du corps = 4 mm ; longueur de l'aile antérieure = 3,75 mm ; longueur de la tarière = 0,5 mm, mesurée à partir de son insertion sur la partie ventrale de l'abdomen, mais, mesurée à partir de la pointe de l'abdomen, elle n'a que 0,25 mm.

♂. Longueur du corps = 4 mm ; longueur de l'aile antérieure = 3,75 mm.

♀. Le corps est couvert de poils blanchâtres, courts, épais et uniformément répandus. Seulement sur le segment intermédiaire ils sont un peu plus longs. Le tégument est coriace, dépourvu de points évidents. La tête est transversale, rétrécie derrière les yeux. Le clypeus est convexe, légèrement différencié de face, avec les fossettes latéro-basilaires petites et assez profondes, à la marge apicale arrondie. La face est transversale

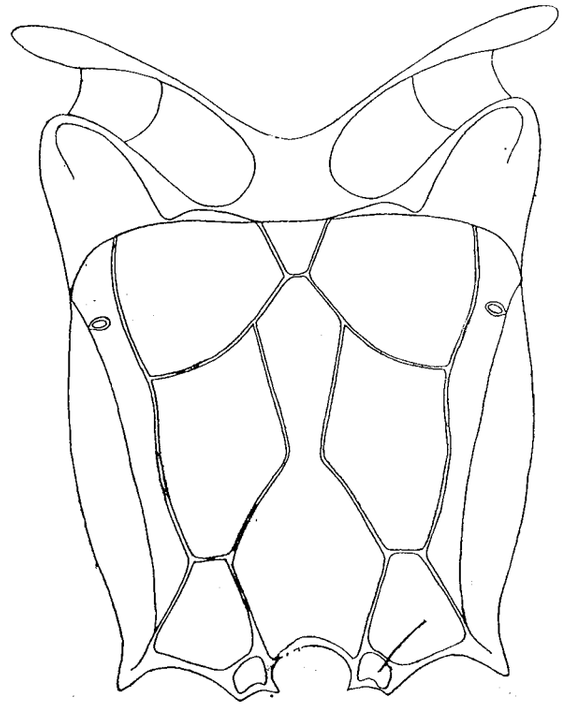


Fig. 2. — Segment intermédiaire de *Nepiera moldavica* nov. sp. ♀, vu sur la partie dorsale. (Original)

à peu près plane. Les tempes sont un peu enflées. Le vertex présente dans la partie postérieure une dépression médio-longitudinale évidente, laquelle s'étend jusqu'à la côte occipitale. Les mandibules sont un peu

rétrécies à la pointe, à dents égales. Les joues sont approximativement aussi longues que la base des mandibules. Les antennes sont filiformes, un peu rétrécies à la pointe. Les fouets sont formés de 26 articles chacun.

Le thorax est un peu plus long que haut. Les sillons parapsideaux font défaut. Le sillon transversal des propleures présente des côtes, plus ou moins longitudinales, assez fortes. Les épomies font défaut. Les épicnémies sont évidentes, mais relativement courtes. L'écusson est un peu convexe, dépourvu de côtes sur ses côtés. Le segment intermédiaire est complètement aréolé, à côtes fines. L'aire supéromédiane est plus longue que large, à peu près hexagonale, ouverte postérieurement (fig. 2). Ses stigmates sont petits, ovales. Les pattes antérieures et moyennes sont faibles, tandis que les postérieures sont plus fortes et plus longues. Les tibias postérieurs présentent des épines minces et éparses dans la partie extérieure, à éperons presque égaux entre eux et aussi longs que la moitié du métatarse. Le nervulus est longuement postfurcalis. L'aréole est sessile (fig. 3 A). Le nervellus est à peu près entier. La discoidella fait défaut.

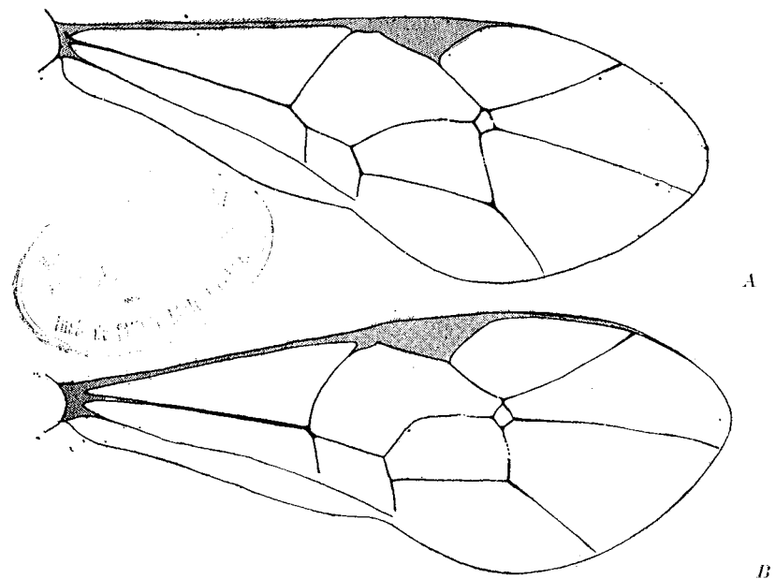


Fig. 3. — Aile antérieure droite : A. de *Nepiera moldavica* nov. sp. ♀, B. de *Nepiera collector* Thunb., ♀. (Original)

L'abdomen est un peu plus long que la tête et le thorax pris ensemble, aplati un peu latéralement à partir de la base du troisième segment. Le premier segment abdominal est approximativement aussi long que les hanches et les trochanters postérieurs pris ensemble. Le deuxième segment est beaucoup plus long que large au bord postérieur, à thyridii évidents. Le troisième segment est aussi long que large, mais le reste des segments sont transversaux.

La couleur fondamentale du corps est noire, avec les palpes, les mandibules sauf les dents, les tegulae, les tegululae, les hanches et les

trochanters antérieurs jaunes blanchâtres ; les callosités humérales sont rouges. Tous les fémurs sont rouges. Les tibias antérieurs et moyens sont rouges, à la partie extérieure un peu blanchâtre. Les tibias postérieurs sont noirâtres, avec la partie intérieure rouge clair au milieu. Les tarses antérieurs et moyens sont rouges, les postérieurs en sont noirâtres, à la partie apicale un peu plus clairs. La marge postérieure des segments abdominaux 1—2 et les côtés des segments 3—7 sont rouges. La plica ventralis est jaunâtre.

♂. Le mâle ressemble à la femelle tant en ce qui concerne les caractères morphologiques externes que la couleur.

Nepiera moldavica nov. sp. ressemble à *Nepiera collector* Thunb. de laquelle elle se distingue par la forme de l'aréole (fig. 3 A, B) et par la couleur des fémurs et des tibias postérieurs, des callosités humérales, etc. Cette espèce ressemble aussi à *Nepiera proxima* Perk., mais elle se distingue de cette dernière par l'aréolation complète du segment intermédiaire, surtout par l'aire supéro-médiane distinctement délimitée par des côtes dans toute son étendue et par la côte latérale évidente ; tandis que chez *Nepiera proxima* Perk. l'aire supéro-médiane ne se distingue pas bien dans la moitié postérieure, et la côte latérale fait presque complètement défaut.

IV. Le genre **Diadegma** Förster, 1868.

4. **Diadegma gibbula** Brischke, 1880, ♀♂.

8 ♀♀ et 1 ♂, éclos de chrysalides de *Plutella maculipennis* Curt., dans les années : 1967—1970, récoltés à Cristeşti, département de Jassy et à Vereşti, département de Suceava.

Cette espèce a été obtenue aussi par des cultures de *Solenobia triquetrella* F.R. (2).

Répartition géographique : Europe.

L'espèce *Plutella maculipennis* Curt. est mentionnée maintenant par nous pour la première fois comme hôte pour cette espèce.

Espèce nouvelle pour la faune de la Roumanie.

D. Section des TRACHYSPHYROIDAE Constantineanu, 1970. (Section des CRYPTOIDAE Dalla Torre, 1901).

Sous-famille des ***PHYGADEUONTINAE*** Dalla Torre, 1902.

V. Le genre **Phygadeuon** Gravenhorst, 1829.

5. **Phygadeuon trichops** Thomson, 1884, ♀♂.

3 ♀♀ et 1 ♂, éclos le 12, 13,23 et 24.X.1970 de pupariums de *Chortophila brassicae* Bouché, récoltés le 27.IX.1970 à Burdujeni, départe-

ment de Suceava ; 1 ♀, éclose le 8.XII.1969, dans des conditions de laboratoire, d'un puparium de *Hylemyia antiqua* Meig., récolté le 21.XI.1969 à Fălticeni, département de Suceava.

Nous mentionnons maintenant pour la première fois dans la science les espèces : *Chortophila brassicae* Bouché et *Hylemyia antiquie* Meig. comme hôtes pour cette espèce.

Répartition géographique : Europe.

Espèce nouvelle pour la faune de la Roumanie.

6. **Phygadeuon geniculatus** Kriechbaumer, 1892, ♂.

1 ♂, éclos le 13.X.1970 d'un puparium de *Chortophila brassicae* Bouché, récolté le 27.IX.1970 à Burdujeni, département de Suceava.

Nous mentionnons pour la première fois dans la science l'espèce *Chortophila brassicae* Bouché comme hôte pour cette espèce.

Répartition géographique : Europe.

Espèce nouvelle pour la faune de la Roumanie.

Sous-famille des ***HEMITELINAE*** Dalla Torre, 1902.

VI. Le genre **Hemiteles** Gravenhorst, 1829.

7. **Hemiteles moldavicus** nov. sp., ♀.

L'holotype ♀, éclos le 29.VIII.1970 d'une chrysalide de *Microplitis spinolae* (Nees), apparue d'une chenille de *Autographa* (*Plusia*) *gamma* L., récoltée à Adjudul Vechi, département de Vrancea, le 18.VIII.1970, leg. Gh. Mustață (dans la collection Mihai I. Constantineanu).

♀. Longueur du corps = 4 mm ; longueur de l'aile antérieure = 3,5 mm ; longueur de la tarière = 0,75 mm.

♀. La tête est transversale, rétrécie d'une manière arrondie derrière les yeux. Le clypeus est relativement grand, convexe, différencié de la face par un fossé large, assez profond, à la marge antérieure tronquée. Les fossettes latéro-basilaires du clypeus sont grandes, peu profondes. La face et le clypeus sont pourvus de poils blanchâtres, relativement longs. Le front est convexe, lisse et luisant. Le vertex est court, lisse et luisant. Les tempes sont courtes, un peu enflées. Les joues sont plus courtes que la base des mandibules. Les antennes sont filiformes, ayant approximativement la longueur du corps, très peu rétrécies à la base des fouets, qui sont formés de 24 articles chacun. Le scapus est beaucoup excavé dans la partie externo-apicale. Le postannellus a la longueur de la partie inférieure du scapus.

Le thorax est plus long que haut, pourvu de poils longs et blanchâtres surtout dans la moitié postérieure. Les sillons parapsideaux sont distincts, assez longs. Le mésonotum et l'écusson sont lisses et luisants. Les

mésopleures sont aussi lisses et luisantes, pourvues d'un fossé longitudinal chacune, un peu oblique, large et profond. Les épomies sont indiquées dans la moitié inférieure seulement. Les épicnémies sont complètes, délicates, courtes. Les sternules sont évidents et longs. Le mésosulcus est distinct, étroit et profond, ouvert postérieurement. Le segment intermédiaire est complètement aréolé, pourvu de côtes délicates. L'aire supéro-médiane est plus longue que large (fig. 4). Le nervulus est interstitialis, très peu postfurcalis. La nervure parallèle s'insère au-dessus du milieu de la marge extérieure de la cellule brachiale (fig. 5). L'angle inféro-extérieur de la cellule brachiale est aigu. La nervure disco-cubitale présente un rudiment de ramellus. Le nervellus est postfurcalis, coudé relativement beaucoup au-dessous de sa moitié. La discoidella est distincte surtout dans la partie intérieure (basilaire). Les pattes sont relativement sveltes.

L'abdomen est fusiforme, luisant. Le premier segment est étroit, trois fois plus long que large dans la partie postérieure, lisse et luisant. Le deuxième segment est pyramidal, avec la pointe dirigée vers la partie antérieure, approximativement aussi long que large postérieurement. Les

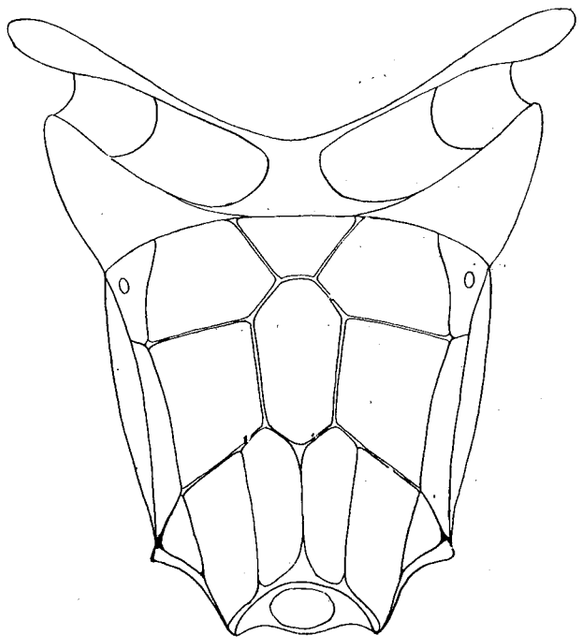


Fig. 4. — Segment intermédiaire de *Hemiteles moldavicus* nov. sp. ♀, vu sur la partie dorsale. (Original)

segments 3—7 sont transversaux. Les segments 2—3 sont pourvus de points épars. Les côtes des segments abdominaux et la partie dorsale des segments postérieurs sont pourvus de points blanchâtres, relativement épaissis.

La couleur fondamentale du corps est noire, avec les palpes blanchâtres. Le milieu des mandibules, les tegulae, les tegululae et les pattes sont rouges. Les onychiums antérieurs et les tarses moyens et postérieurs sont bruns. Le pterostigma est brun. Les valves génitales externes sont noirâtres, mais la tarière est rougeâtre. L'abdomen est noir, avec les segments 2—4 rouges — le quatrième segment à côtés noirs.

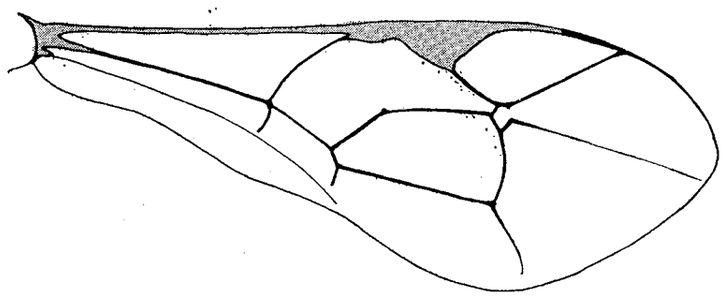


Fig. 5. — Aile antérieure droite de *Hemiteles moldavicus* nov. sp., ♀. (Original)

Hemiteles moldavicus nov. sp. ressemble à *Hemiteles stagnalis* Thoms., surtout par la nervure parallèle, insérée au-dessus du milieu de la marge extérieure de la cellule brachiale. Mais il se distingue de celui-ci par l'angle inféro-extérieur pointu de la cellule brachiale, par la forme beaucoup plus svelte du premier segment abdominal, ainsi que par la couleur différente du corps.

♂ inconnu.

Sous-famille des ***STILPNINAE*** Dalla Torre, 1902.

VII. Le genre **Stilpnus** Gravenhorst, 1829.

8. **Stilpnus gagates** Gravenhorst, 1829, ♀♂.

3 ♀♀ et 1 ♂, éclos le 15, 16 et 19.XII.1970 de pupariums de *Chortophila brassicae* Bouché, récoltés le 14.VIII.1970 à Tîrgu Frumos, département de Jassy ; 1 ♀, éclose le 3.IX.1970 d'un puparium de *Hylemyia antiqua* Meig., récolté le 14.VIII.1970 à Vereşti, département de Suceava.

Écologie. Cette espèce a été obtenue antérieurement par des cultures de : *Pegomyia hyoscyami betae* Curt., *Chortophila brassicae* Bouché, *Ch. floris* Fall. et *Anthomyia radicum* L., d'après W.R. Thomson (1957) et G. Leonardi (1927).

Maintenant nous signalons l'espèce *Chortophila brassicae* Bouché pour la première fois dans la Roumanie comme hôte pour cette espèce.

Répartition géographique ; Belgique et U.R.S.S.

Espèce nouvelle pour la faune de la Roumanie.

CONCLUSIONS

Dans ce travail les auteurs signalent :

I. Deux espèces nouvelles pour la science, à savoir : 1. *Nepiera moldavica* ♀♂ et 2. *Hemiteles moldavicus* ♀.

II. Six espèces nouvelles pour la faune de la Roumanie, à savoir : 1. *Scambus eucosmidarum* Perk., ♀, 2. *Homotropus asyntactus* Schm., ♂, 3. *Diadegma gibbula* Brisch., ♀♂, 4. *Phygadeuon trichops* Thoms., ♀♂, 5. *Ph. geniculatus* Kriechb., ♂ et 6. *Stilpnus gagates* Grav., ♀♂.

BIBLIOGRAPHIE

1. Constantineanu M. I., Pisică C., An. Șt. Univ. « Al. I. Cuza » Iași (seria nouă), Secț. II (Șt. nat.) a. Biol., 1966, *1*, 205—215.
2. Hedwig K., Nachr. der Sammelstelle für Schmarotzerbestimmung, 1950, *24*, 27—42.
3. Leonardi C., *Elenco delle specie degli insetti dannosi e loro parassiti ricordati in Italia fino all'anno 1911, Parte III, Insetti parassiti di altri insetti, Ichneumonidae*, 1927, Modena, 76—136.
4. Perkins J. F., The Entomologist, 1942, *75*, 64—65.
5. Schmiedeknecht O., *Opuscula Ichneumonologica*, 1902—1927, **1—5**, *1—45*, 1—3570, Blankenburg i. Thür.
6. Schmiedeknecht O., *Opuscula Ichneomonologica. Supplement-Band, Neuebearbeitungen*, 1928—1936, *1—25*, Blankenburg i. Thür.
7. Thompson W. R., *A catalogue of the Parasites and Predators of Insect Pests. Ssect. 2. Host Prasites Catalogue. Part 4. Host of the Hymenoptera (Ichneumonidae)*, 1957, Ed. The Commonwealth Institute of Biological Control, Ottawa, 332—561.
8. Townes H., Momoi S. and Townes M., Mem. Amer. Ent. Inst., 1965, **5**, 1—661.

Reçu le 11 septembre 1971

Université « Al. I. Cuza »
Jassy

CONCEPTIONS, RÉALISATIONS ET PERSPECTIVES DANS LA LÉPIDOPTÉROLOGIE

PAR

EUGEN V. NICULESCU

In this paper the author analyses various conceptions existing in Lepidopterology in the field of systematics and phylogeny. For the contemporary period, the extreme splitters viewpoint is criticized, as it results only in an overburdened nomenclature, without any advantage for science. Positive aspects of contemporary Lepidopterology are also emphasized in a suggestive table, summing up the most important achievements of the last 50 years. The work ends with the specification of further tasks incumbent on present-day and future lepidopterologists, to cover existing gaps.

La systématique, comme tout autre science, a évolué, elle aussi, incessamment, au cours de ses deux siècles et plus d'existence. Non seulement on peut parler de la « systématique du XIXème siècle », mais aussi considérer dans ce siècle plusieurs périodes se distinguant des précédentes par d'autres méthodes de recherche, d'autres conceptions sur l'espèce, le genre, la classification, etc.

Dans les 6 dernières décennies du XVIIIème siècle il y avait une seule systématique puisqu'il n'existait qu'une seule conception sur l'espèce et le genre et qu'une seule méthode de recherche. Tout le monde avait adopté la systématique de Linné basée sur la conception fixiste de l'espèce, sur l'idée très primitive de la classification de l'ordre *Lepidoptera* en trois genres seulement, sur la méthode simpliste de la description des espèces uniquement d'après leur habitus. Les systématiciens ne se posaient pas de « problèmes » à résoudre comme aujourd'hui. Ils n'étaient pas préoccupés par le genre où placer la nouvelle espèce décrite, étant donné que, pour l'ensemble des papillons diurnes, il n'y avait qu'un

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, N° 2, p. 97–106, BUCAREST, 1972

seul genre : *Papilio*. Leur seule préoccupation était de chasser les papillons, les étaler et leur donner un nom spécifique.

La systématique linnéenne a duré, pour les Rhopalocères, jusqu'en 1801 quand F. Paula Schrank a érigé les genres *Pieris*, *Cupido*, *Maniola* et *Erynnis*. Plus tard M. Kluk (1802) a décrit les genres *Nymphalis* et *Plebejus* et J. Fabricius (1807) *Apatura*, *Limenitis*, *Vanessa*, *Melitaea*, *Argynnis*, *Lycaena*, etc. La systématique de Linné a ainsi cessé d'exister en exclusivité : elle a commencé à être remplacée par la systématique de J. Fabricius, P. Latreille, W. Leach, J. Hübner, J. Boisduval, etc. [26]. A été changée aussi la méthode de recherche étant donné que Latreille avait découvert de nombreux caractères morphologiques qui étaient, cette fois-ci, pris en considération : la conformation des pattes, des antennes, de la trompe chez l'imago, des pattes de la chenille, etc. et Boisduval avait montré une série de particularités des chrysalides. Une idée nouvelle sur la classification était apparue ainsi que de nouveaux taxa outre l'espèce et le genre : la famille et la tribu (P. Latreille).

Comme toujours quand paraît du « nouveau », l'« ancien » continue à durer encore quelque temps, puisque celui qui s'est accoutumé à une certaine conception et méthode de travail, le plus souvent, ne veut pas les abandonner. Ainsi à une certaine époque ont existé en Europe « deux systématiques » : celle de Linné qui était encore utilisée et la « nouvelle systématique », basée sur les idées nouvelles de Fabricius, Latreille, Leach, Hübner, Boisduval, etc. Cette dernière est decenue, elle aussi, désuète à un moment donné. Ainsi P. Rambur avait découvert le plus important caractère taxonomique pour la détermination des espèces : l'armure génitale. Malheureusement l'idée géniale de Rambur n'a pas été adoptée et pendant 40 années elle a été ignorée. Au contraire, l'idée de Herrich-Schäffer d'utiliser la nervulation comme un critère de genre a été plus heureuse et a eu de nombreux adeptes.

Mais les systématiciens du XIXème siècle n'ont pas brillé par leurs recherches morphologiques. Leur grand mérite est tout autre. Ils ont exploré une grande superficie du Globe et ont rassemblé un riche matériel lépidoptérologique, ces vastes collections constituant le « fonds d'or » de la Lépidoptérologie. La plupart des espèces et des genres connus aujourd'hui ont été décrits au cours du XIXème siècle. Mais les intrépides chercheurs sur le terrain ont eu, en même temps, un grand défaut : celui d'ignorer l'étude de l'armure génitale et, en général, de la morphologie, ce qui a eu comme conséquence un nombre impressionnant d'erreurs systématiques. Le « nouveau » a pénétré avec une très grande difficulté. On disait que c'était un sacrilège de « mutiler » un insecte et d'endommager l'esthétique d'une collection. La collection n'était pas un « moyen » mais un « but ». Ces collectionneurs non seulement ne laissaient pas disséquer leurs bêtes, mais n'accordaient que mépris aux « génitalistes » avides de « fabriquer » de nouvelles espèces pour le « seul plaisir de donner des noms » (H. Marion, 1954).

Dans les dernières décennies du XIXème siècle et les premières du XXème siècle s'est introduite une nouvelle catégorie taxonomique : la superfamille, et l'étude de l'armure génitale avait acquis un développement prometteur par les recherches de F. Buchanan White, P. H. Gosse, V. Klinkhardt, L. Poljanec, H. Stitz, H. Stichel, E. Zander, W. Petersen,

F. N. Pierce, G. P. Bethune-Baker, T. A. Chapman, N. Ia. Kusnezov, etc. Malgré ces recherches précieuses, l'étude de l'armure génitale n'était pas généralisée et l'« ancienne systématique » du XIXème siècle était encore très répandue [26]. Les lépidoptéristes de cette période étaient, pour la plupart, réfractaires acharnés à l'étude des genitalia ; toutefois ils ont contribué substantiellement au développement de la Lépidoptérologie. Certains ont décrit un grand nombre de nouveaux taxa : A. R. Grote, H. G. Dyar, G. F. Hampson, O. Staudinger, Ch. Oberthür, A. Caradja (plus d'un millier), Ed. Meyrick (plus de 16 000) ; d'autres ont élaboré des classifications : A. S. Packard (1895), H. Dyar (1902), N. Ia. Kusnezov (1915), Ed. Meyrick (1928), ou ont publié d'importants catalogues : O. Staudinger et H. Rebel, et des Faunes : A. Seitz, A. Spuler.

La Lépidoptérologie moderne doit beaucoup à Staudinger, Rebel, Seitz et Spuler, mais en même temps ils sont responsables, par leurs travaux, de la propagation, dans le monde entier, d'un grand nombre d'erreurs systématiques. Celles-ci sont de plusieurs sortes :

1. L'omission d'un nombre de bons genres comme *Iphiclides* Hbn., *Pontia* F., *Nymphalis* Kluk, *Inachis* Hbn., *Aglais* Dalm., *Boloria* Moore, *Cupido* Schrank, *Syntarucus* Btl., *Scolitantides* Hbn., *Philotes* Scudder, *Glaucopsyche* Scudder, *Plebejus* Kluk, etc.

2. L'utilisation de certains noms de genres non valables : *Thais* F. au lieu de *Zerynthia* Ochs., *Leucochloë* Röber au lieu de *Pontia* F., *Melanargia* Meig. au lieu d'*Agapetes* Billb., *Chrysophanus* Hbn. au lieu de *Lycaena* F., etc.

3. La répartition de certaines espèces dans des genres autres que ceux où il faut, justement, les inclure : *atalanta* L., *cardui* L., *indica* Herbst ont été incluses dans le genre *Pyrameis* au lieu de *Vanessa* ; *io* L., *urticae* L., *polychloros* L., *antiopa* L., réparties dans le genre *Vanessa* au lieu des genres *Inachis*, *Aglais*, *Nymphalis* ; *nogelii* Her. Schäffer, *ballus* F., *mauritanicus* Luc., etc. dans le genre *Thestor* (sud-africain)au lieu de *Tomares* (palaearctique) ; *argus* L., *orion* Pall., *baton* Bgstr., *alexis* Poda, etc. dans le genre *Lycaena* au lieu des genres *Plebejus*, *Scolitantides*, *Philotes*, *Glaucopsyche*, etc.

La période comprenant les deux premières décennies du XXème siècle pourrait être nommée une période « indécise », une lutte continue entre les anciens systématiciens hostiles à l'étude de l'armure génitale et les nouveaux systématiciens propagateurs d'une nouvelle méthode de recherche et d'une nouvelle conception.

Une autre caractéristique de cette période est aussi la conception de l'espèce monotypique — la même sur toute son aire de distribution — — héritée toujours du dernier siècle, ainsi qu'une diversité d'opinions en ce qui concerne les formes subspécifiques toutes mélangées, pêle-mêle, et confondues sous le nom de variété [31].

A partir approximativement de la 3ème décennie du XXème siècle a lieu dans la Lépidoptérologie mondiale un revirement dans la systématique dû à la généralisation totale de l'étude des genitalia qui embrasse tous les groupes de Lépidoptères.

On réalise de nombreux ouvrages capitaux d'une haute tenue scientifique, on élabore de nouvelles classifications de l'ordre où la superfamille est un taxon obligatoire et s'entreprennent des recherches phylogénétiques

destinées à intégrer plus exactement les familles dans le système. Ce sont les travaux de Turner et Tillyard, C. Börner, et plus tard ceux de J. Bourgogne [4], S. G. Kiriakoff [16], E. V. Niculescu [27], [31], [32], [33], etc.

Malheureusement ces intéressantes réalisations sont assombries par une conception erronée de la notion de genre qui a abouti à la fragmentation à outrance des genres jusqu'alors acceptés. Le phénomène est passé aussi dans le domaine de l'espèce pour atteindre, actuellement, les taxa plus élevés (familles et superfamilles). A la multiplication exagérée des genres a contribué le « déterrement » de certains anciens genres érigés au début du XIXème siècle alors que l'armure génitale n'était pas connue. Ainsi, depuis les genres comprenant 200, 100 ou 50 espèces on est arrivé aux genres de 5, 4, 2 espèces ou même aux genres monotypiques. Cette conception a été adoptée, malheureusement, par la plupart des lépidoptéristes. A l'extrême opposé se trouvent les lépidoptéristes « conservateurs » qui utilisent encore la systématique et la nomenclature de Staudinger et Seitz. En opposition avec les lépidoptéristes « extrémistes » (splitters — diviseurs et lumpers — réunisseurs) il y a un nombre de « modérés » qui ne se dirigent plus d'après la systématique de Staudinger et Seitz, mais n'adoptent pas non plus le point de vue extrême-diviseur. Ils ont protesté véhémentement contre les excès des diviseurs qui veulent « créer un nouveau genre d'après un poil de plus sur l'armure génitale » et ils ont adopté une position intermédiaire. Parmi ceux-ci nous mentionnons S. Le Marchand, G. Warnecke, S. G. Kiriakoff [16], J. Bourgogne (partiellement), J. F. Gates Clarke, Ch. Boursin, B. Alberti [1], H. Marion [18], H. Stempffer, J. Dabrowski [8], T. Shirôzu, H. Malicky, E. V. Niculescu [20], [21], [23], [25], [28], [32]. Parmi les lépidoptéristes « splitters » qui ont contribué le plus à la propagation du point de vue diviseur nous mentionnons J. Tutt, T. Reuss, H. Beuret, L. Higgins [14], [15], W. H. T. Tams, B. C. S. Warren [37], J. G. Franclemont, W. Forster [12] et surtout R. Verity [36]. Ce dernier a publié un ouvrage grandiose en 5 volumes sur les Rhopalocères d'Italie. Sa classification et nomenclature ont été adoptées dans tous les pays d'Europe. Mais personne n'a vérifié les données morphologiques et les interprétations de Verity. En étudiant tout spécialement la morphologie des Rhopalocères et toute l'œuvre de Verity, nous avons constaté qu'il a transféré, arbitrairement, les caractères spécifiques au niveau générique, en adoptant presque tous les genres de Scudder, Tutt, Reuss, Hemming, Warren, Higgins, etc ; bien plus, il a créé, en outre, de nouveaux genres, en se plaçant toujours sur les positions les plus avancées de ce point de vue extrême-diviseur. Quoique sa conception soit aujourd'hui acceptée par la plupart des lépidoptéristes, nous sommes toutefois d'avis qu'elle est erronée et la systématique de l'avenir sera celle préconisée par nous et par nos collègues « modérés » mentionnés ci-dessus.

Pour préciser mieux encore notre position sur ce problème, nous présentons, ci-joint, une liste des genres de quelques familles qui, à notre avis, ne sont pas valables.

Pterourus Scop., *Princeps* Hbn., *Toas* Schwainson, *Achivus* Kirby, *Eques* Kirby, *Thais* F., *Allancastria* Bryk, *Tadumia* Moore, *Lingamius* Bryk, *Koramius* Moore, *Eukoramius* Bryk, *Kailasius* Moore, *Leucophasia* Steph., *Leptidia* Scudder, *Azalais* Grote, *Tetracharis* Grote, *Leuconea* Donzel, *Futuronerva* Bryk, *Mancipium* Hbn., *Leucochloë* Röber, *Scolidoneura* Btl., *Eriocolias* Watson, *Coliastes* Hemming, *Goneptera* Billberg, *Rhodocera* Boisduval et Leconte, *Earina* Speyer, *Aeola* Billberg, *Apaturina* Sodovski, *Eugonia* Hbn., *Grapta* Kirby, *Euvanessa* Scudder, *Pyrameis* Hbn., *Cinclidia* Hbn., *Mellicta* Billberg, *Argyrea* Billberg, *Argyreus* Scopoli, *Issoria* Hbn. *Argyronome* Hbn., *Damora* Nordman, *Speyeria* Scudder, *Rathora* Moore, *Fabriciana* Reuss, *Prodryas* Reuss, *Mesoacidalia* Reuss, *Kükenthaliella* Reuss, *Prokükentaliella* Reuss, *Profabriciana* Reuss, *Proacidalia* Reuss, *Mesodryas* Reuss, *Protodryas* Reuss, *Pandoriana* Warren, *Childrena* Hemming, *Chrysophanus* Hbn., *Heodes* Dalman, *Palaeoloweia* Vrty, *Loweia* Tutt, *Sarthusia* Vrty, *Hyrcanana* Bethune-Baker, *Thersamonia* Vrty, *Palaeochrysophanus* Vrty, *Rumicia* Tutt, *Raywardia* Tutt, *Langia* Tutt, *Cosmolyce* Toxopeus, *Celastrina* Tutt, *Tiora* Evans, *Apelles* Hemming, *Turanana* Bethune-Baker, *Lycaeides* Hbn., *Eumedonia* Forster, *Pseudoaricia* Brt., *Vacciniina* Tutt, *Lysandra* Hemming, *Meleageria* Sag., *Agrodiaetus* Hbn., *Plebicula* Higgins, *Latiorina* Tutt, *Nomiades* Hbn., *Uranops* Hemming, *Hirsutina* Tutt, *Quercusia* Vrty, *Strymonidia* Tutt, *Thecliola* Strand, *Chattendenia* Tutt, *Tuttiola* Strand, *Nordmania* Klug, *Lopinga* Moore, *Lasiommata* Westwood, *Amecera* Btl., *Kirinia* Moore, *Melanargia* Meigen, *Arge* Hbn., *Halimede* Obth., *Epimede* Obth., *Ledargia* Obth., *Chortobius* Dunning et Pickard, *Phorcis* Hbn., *Epigea* Hbn., *Syngea* Hbn., *Marica* Hbn., *Gorgo* Hbn., *Oreina* Westw., *Epinephele* Hbn., *Epinephila* Steph., *Chionobas* Bsdv. *Brintesia* Frhst., *Minois* Hbn., *Arethusana* de Lesse, *Chazara* Moore, *Eumenis* Hbn.., *Melania* Sodowski, *Thanaos* Bsdv., *Syrichtus* Bsdv., *Scelotrix* Rbr., *Powellia* Tutt., *Sloperia* Tutt, *Augiades* Hbn., *Pamphila* F., *Urbicola* Tutt, *Nissoniades* Hbn., *Nemeobius* Steph., *Agrumenia* Hbn., *Mesembrynus* Hbn., *Silvicola* Bg., *Lycastes* Hbn., *Hyala* Bg., *Hesychia* Hbn., *Santolinophaga* Bg., *Peucedanophila* Bg., *Lictoria* Bg., *Peristygia* Bg., *Coelestis* Bg., *Agrumenoidea* Hol., *Thermophila* Hbn., *Polymorpha* Bg.

Une autre caractéristique de la Lépidoptérologie de cette période est aussi l'adoption de la notion d'espèce polytypique, l'espèce étant cette fois considérée comme formée de plusieurs races répandues sur l'aire géographique générale de l'espèce. Cette conception a été embrassée, avec beaucoup d'intérêt, par tous les lépidoptéristes, ce qui a eu comme conséquence l'introduction dans la systématique d'une nomenclature trinominale pour exprimer la variation géographique. Malheureusement cette importante conquête de la biologie moderne a été « dénaturée » par certains lépidoptéristes qui ont « pulvérisé » la race de même qu'on l'avait fait, antérieurement, avec l'espèce. Presque chaque race a été divisée en d'autres 5—10 races, parfois chaque population étant considérée comme une race distincte recevant ainsi un nom nouveau. Dans cette direction ont travaillé activement H. Fruhstorfer, F. Bryk, C. Eisner, F. Dujardin [1] et surtout R. Verity. Les groupes le plus affectés par la « manie des races » sont les *Parnassiinae*, les *Melitaea*, *Argynnis*, *Zygaena*, divers genres de *Lycaenidae*, etc.

[1] Cet auteur, réunisseur quand il s'agit des genres (*Lycaenidae* [8]), est diviseur à propos des races (*Parnassiinae*, *Zygaeninae* [7]).

Excepté ces aspects négatifs de la Lépidoptérologie contemporaine, il faut souligner en même temps ses aspects positifs et la voie ascendante de notre science.

Les 50 dernières années ont été publiées un grand nombre de monographies, révisions, Faunes, catalogues, outre la description d'un grand nombre d'espèces et de genres nouveaux. Quoique certains de ceux-ci tombent rapidement en synonymie, beaucoup sont toutefois valables et font augmenter le trésor lépidoptérologique mondial. Le tableau ci-dessous est destiné à montrer ces grandes réalisations des spécialistes qui ont publié des monographies et diverses révisions.

MONOGRAPHIES ET RÉVISIONS

Eriocraniidae : J. Heath, S. Toll, P. Viette.
Micropterygidae : J. Heath, S. Toll, P. Viette.
Hepialidae : R. Agenjo *(Hepialus)*, F. Daniel, S. Toll, P. Viette.
Stigmellidae : B. P. Beirne, E. M. Hering, T. N. Freeman, J. Klimesch, N. L. Wolf.
Incurvariidae : D. R. Davis *(Prodoxinae)*.
Adelidae : J. Heath, M. Okano, T. Yasuda.
Tischeriidae : S. Toll.
Cossidae : H. K. Clench, F. Daniel, P. Viette.
Psychidae : J. Bourgogne, D. R. Davis, I. V. Kozantschikow, W. Sauter, L. Sieder, Kôji-Yano.
Tineidae : H. Amsel, A. Diakonoff, L. Gozmany, G. Petersen, A. K. Zaguleaev, I. Căpuşe.
Acrolophidae : F. F. Hasbrouck.
Lithocolletidae : F. Gregor, E. Janmoulle *(Ornix)*, T. Kumata, D. Povolny, L. Vari.
Phyllocnistidae : E. F. Martînova.
Yponomeutidae : G. Friese, S. Moriuti.
Argyresthiidae : G. Friese.
Stenomidae : W. D. Duckworth.
Acrolepiidae : S. Moriuti, J. Klimesch.
Plutellidae : G. Friese, S. Moriuti.
Gelechiidae : T. N. Freeman, L. Gozmany, A. J. T. Janse, J. Klimesch, S. Le Marchand, D. Povolny, K. Sattler, I. Svensson *(Bryotropha)*, N. L. Wolff.
Momphidae : R. W. Hodges *(Walshiinae)*, F. Kasy, T. Riedl.
Oecophoridae : J. F. Gates Clarke, H. J. Hannemann *(Depressaria)*, S. Toll, P. Viette *(Depressaria)*.
Scythrididae : N. L. Wolff.
Ethmiidae : K. Sattler.
Elachistidae : T. Kumata.
Eupistidae : W. Hackman, J. Klimesch, J. Suire, S. Toll.
Aegeriidae : G. P. Engelhardt, M. Rae Mackay (chenilles), C. Naumann, E.V. Niculescu (en collaboration avec A.Popescu-Gorj et Al. Alexinschi), Z. Schnaider, R. Schwarz, P. Viette.

Tortricidae : H. Amsel, P. Benander, G. A. Graaf Bentinck, J. F. Gates Clarke *(Olethreutinae)*, A. S. Danilewski, W. Deurs, A. M. Diakonoff, M. I. Falcovici, T. N. Freeman, H. J. Hannemann, V. I. Kusnezov, M. Rae Mackay (chenilles), N. S. Obraztsov, M. Okano, M. Peiu et I. Nemeş *(Ancylis)*, A. Powell, J. Razowski, S. Toll, N. L. Wolff, T. Yasuda.
Cochylidae : M. I. Falcovici, H. J. Hannemann, J. Razowski.
Carposinidae : D. R. Davis, H. J. Hannemann, J. Razowski.
Zygaenidae : A. Bayard *(Procris)*, H. Burgeff, B. Alberti, R. Agenjo *(Procris)*, J. Dabrowski, O. Holik, L. Sheljuzko.
Pterophoridae : H. Amsel, L. Bigot, R. Schwarz, Kôji-yano.
Galleriidae : P. E. S. Whalley.
Pyralidae : H. Amsel, S. Bleszynschi *(Crambinae)*, H. W. Capps *(Leucinodes, Loxostege)*, H. J. Hannemann, C. Heinrich *(Phycitinae)*, E. Janmoulle *(Homoeosoma)*, Al. B. Klots *(Crambinae)*, G. de Lattin *(Crambinae)*, H. Marion, E. L. Martin, E. Munroe, M. Okano, U. Roesler *(Phycitinae)*.
Thyrididae : E. S. Whaley.
Timyridae : L. Gozmany.
Axiidae : H. Reisser.
Drepanidae : A. Watson.
Limacodidae : H. K. Clench.
Geometridae : S. Bleszynschi, H. W. Capps, G. Hagett (chenilles), C. Herbulot, E. de Laever, M. Rae Mackay (chenilles), J. Mc Dunnough *(Eupithecia, Hydriomena)*, W. C. McGuffin (chenilles), H. Reisser, F. H. Rindge *(Glena, Glaucina, Melanolophia* etc.*)*, F. Urbahn, Seda A. Vardikian, E. P. Wiltshire.
Lymantriidae : R. Agenjo, I. V. Kozhantschikov.
Noctuidae : R. Agenjo (*Catocala*), E. Berio, Ch. Boursin *(Trifinae)*, C. Dufay *(Quadrifinae, Plusiinae)*, D. C. Ferguson, W. T. M. Forbes, J. G. Franclemont, G. Hagett (chenilles), D. F. Hardwick, A. S. Kostrowicki *(Agrotinae, Melicleptriinae)*, I. V. Kozhantschikov *(Agrotinae)*, G. de Lattin, W. C. McGuffin (chenilles), P. Viette *(Amphpyirinae* et *Melicleptriinae)*.
Agaristidae : S. G. Kiriakoff.
Arctiidae : Birket — Smith *(Lithosiinae)*, J. G. Franclemont, M. Okano, H. de Toulgoët, L. Travassos Filho, A. Watson.
Ctenuchidae : F. Daniel, P. Griveaud, N. S. Obraztsov, L. Travassos.
Cochlidiidae : J. D. Dabrowski.
Notodontidae : F. Daniel, J. G. Franclemont, S. G. Kiriakoff, M. Okano.
Thaumetopoeidae : R. Agenjo.
Thyretidae : S. G. Kiriakoff.
Bombycoidea : P. C. Rougeot.
Attacidae : P. Griveaud, Cl. Lemaire, P. C. Rougeot.

Lasiocampidae : Yves de Lajonquière, P. C. Rougeot, F. W. Stehr et E. F. Cook *(Malacosoma)*, P. Viette.
Sphingidae : P. Griveaud.
Hesperiidae : B. Alberti, L. Berger, P. R. Ehrlich, M. Guillaumin, G. Kaufmann, O. Masani, M. Ogata, J. Picard, P. Viette, B. C. S. Warren.
Papilionoidea : R. F. D'Almeida, A. S. Corbet et H. M. Pendlebury, T. Iwase, M. Krzywicky, A. I. Kurenzov, S. Murayama, M. Okana, C. F. dos Passos, W. J. Reinthal, N. D. Riley, T. Shirôzu, R. Verity.
Danaidae : R. F. D'Almeida, R. Paulian.
Riodinidae : N. H. Bennett, W. S. McAlpine, P. Rebillard.
Lycaenidae : N. H. Bennett, H. Beuret, H. K. Clench, W. D. Field *(Calystryma, Calicopis)*, O. Hoegh-Guldberg *(Aricia)*. Nabokov, W. Sauter, T. Shirôzu (Theclini), H. Stempffer, L. J. Toxopeus.
Satyridae : A. Avinov et W. R. Sweadner *(Karanasa)*, M. Condamin, W. Forster, H. de Lesse *(Lethe*, etc.*)*, B. C. S. Warren *(Erebia)*, C. W. Wyatt, A. Popescu-Gorj *(Erebia)*.
Nymphalidae : L. P. Grey, L. G. Higgins *(Melitaeinae)*, E. V. Niculescu, G. Van Son, B. C. S. Warren *(Argynninae)*.
Pieridae : L. A. Berger *(Colias)*, G. Bernardi *(Pierinae)*, J. Herrera et W. D. Field *(Theochila* et *Tatochila)*, Al. B. Klots, E. V. Niculescu, G. Van Son.
Papilionidae : L. A. Berger, C. Eisner *(Parnassiinae)*, E. V. Niculescu, R. Roy, G. Van Son, A. Villiers.

De cette liste on voit que presque tous les groupes ont été abordés et qu'un grand nombre de genres et familles ont été révisés sur de nouvelles bases. Outre ces travaux, ont été publiés divers traités, ouvrages de détermination, catalogues, guides, etc. très utiles aussi. Dans cette catégorie nous mentionnons en premier lieu le grand ouvrage en 3 volumes de E. M. Hering [13] ainsi que l'ouvrage de Bergmann [3] en 7 volumes. Les généralités sur les Lépidoptères sous leurs aspects : morphologique, phylogénétique, biologique, etc. se trouvent dans les travaux de J. Bourgogne [4], W. Forster et Th. Wohlfahrt [12] et surtout de E. V. Niculescu et Fr. König [33]. Un utile glossaire des termes morphologiques des genitalia a été publié sous la rédaction de S. L. Tuxen [35]. Dans le domaine de la Zoogéographie nous mentionnons le précieux travail de G. de Lattin et dans celui de la biologie l'intéressant livre d'Al. Klots *Vie et mœurs des papillons*. De même sont intéressants et utiles, surtout pour les débutants, les travaux de M. Koch, L. Higgins [15], Al. I. Kurenzov [17] et W. B. L. Manley [19]. Enfin, la grande œuvre *Microlepidoptera palaearctica* sera le travail le plus vaste et le plus complet sur les Microlépidoptères. Jusqu'à présent ont paru trois volumes : Les *Crambinae* par S. Bleszynschi, les *Ethmiidae* par K. Sattler, et les *Cochylidae* par J. Razovschi.

Les réalisations de la Lépidoptérologie contemporaine sont, par conséquent, nombreuses et de grande portée scientifique. Toutefois il reste encore beaucoup de tâches à accomplir. Si dans certains secteurs les

résultats sont remarquables, dans d'autres nous avons encore beaucoup à faire.

1. Il y a encore un nombre de régions du Globe insuffisamment explorées, donc l'inventaire lépidoptérologique mondial n'est pas achevé.
2. Chez nombre d'espèces l'armure génitale ♂ et surtout ♀ est inconnue.
3. L'étude de l'exosquelette, pour des fins systématiques, est à peine à son début.
4. Il y a un nombre considérable de chenilles, surtout parmi les microhétérocères complètement inconnues et les descriptions faites sur celles déjà connues sont, le plus souvent, superficielles (seulement d'après l'habitus), incomplètes et parfois erronées. Chez un grand nombre de chenilles la structure des pièces buccales, des pattes abdominales et la chêtotaxie sont inconnues. Les recherches sur les œufs et les chrysalides sont aussi tout à fait incomplètes.
5. L'étude écologique et éthologique des chenilles est, également, à son début.
6. Les relations phylogénétiques entre les divers taxa sont insuffisamment connues et souvent très confuses.
7. Il n'y a pas une classification satisfaisante de l'ordre *Lepidoptera*.

Voilà donc une série de tâches qui se présentent aux lépidoptéristes pour les décennies futures. A notre avis, l'élaboration, d'une part, d'une bonne classification et, d'autre part, d'un arbre phylogénétique de l'ordre *Lepidoptera*, sera seulement possible après l'étude complète de l'exosquelette. Cette étude représentera, pour les taxa supérieurs, ce qu'a représenté l'armure génitale pour les taxa inférieurs. En ce qui concerne la conception qui doit diriger la classification de ces derniers, c'est, sans doute, le point de vue réunisseur. Actuellement, la « lutte d'opinions » entre les splitters et lumpers est encore puissante, mais nous sommes convaincus que la « victoire » sera du côté de ces derniers.

BIBLIOGRAPHIE

1. Alberti B., Ber. ü. 8.Wandervers. Deutsch. Entom., 1957, 138.
2. — Mitt. Zool. Mus. Humboldt Univ. Berlin, 1954, **30**, 115–480.
3. Bergmann A., *Die Gross Schmetterlinge Mitteldeutschlands*, 1951–1955, Jena, 1–5.
4. Bourgogne J., *Ordre des Lépidoptères*, in Grassé P. *Traité de Zoologie*, 1951, Mason, Paris, *X*, 1, 174–448.
5. Căpuşe Iosif, *Fauna Republicii Socialiste România, Insecta, Fam. Tineidae*, 1968, **XI**, *9*, Ed. Acad., Bucarest.
6. Caradja A., Acad. Rom. Mem. Secţ. Şt., Seria III, 1925, **3**, 7 : 257–387, pl. 1–2.
7. — Acad. Rom. Mem. Secţ. St., Seria III, 1927, **4**, 8 : 361–428.
8. Dabrowski J. D., Acta. zool. Cracov., 1965, **10**, *2*, 91–196.
9. Dujardin F., Entomops, 1965, *1*, 16–22 ; *2*, 33–64 ;
10. — Entomops, 1969, *15*, 241–244.
11. Eisner Curt, Zool. Med.-Parnassiana Nova, 1954–1960, **I–XXVIII**, Leiden.
12. Forster W. et Wohlfahrt Th. A., *Die Schmetterlinge Mitteleuropas*, 1954–1970, I, II, III, IV, Ed. Franckh, Stuttgart.
13. Hering E. M., *Bestimmungstabellen der Blattminen von Europa einschliesslich des Mittelmeerbeckens und der Kanarischen Inseln*, 1957, 1–648 ; 649–1185 ; III, 1–221, 725 fig.
14. Higgins L. G., Trans. Roy. Ent. Soc. London, 1941, **91**,175–365, 215 fig., 16 pl.
15. Higgins L. G. et Riley N. D., *A Field Guide to the Butterflies of Britain and Europe*, 1970, 380 p., 60 pl. col.
16. Kiriakoff S. G., Lambillionea, 1946, **1**, 3–7.

17. KURENZOV A. I., *Rhopalocères de l'Extrême Orient de l'U.R.S.S.*, ouvrage de détermination, 1970, 163 p., 104 fig., XIV pl. col., Ed. « Science », Leningrad.
18. MARION H., Rev. fr. de Lépidoptérologie, 1954, **13—14**, 181—188.
19. MANLEY W. B. L. and ALLCARD H. G., *A Field Guide to the Butterflies and Burnets of Spain*, 1970, 191 p., 41 pl. col., Classey Hampton.
20. NICULESCU E. V. *Fauna Republicii Populare Române, Insecta, Lepidoptera, Fam. Papilionidae*, 1961, **XI**, *5*, 5—103, 32 fig., IX pl.
21. NICULESCU E. V., *Fauna Republicii Populare Române, Insecta, Lepidoptera, Fam. Pieridae*, 1963, **XI**, *6*, 5—202, 66 fig., XIII pl.
22. — Linneana, Belgica, 1964, Pars III, *3*, 34—45, 4 fig.
23. — Boll. della Soc. Ent. Ital., 1964, **XCIV**, *1—2*, p. 36—39.
24. — Bull. de la Soc. Ent. de Mulhouse, 1964, janvier-février, 1—16.
25. — *Fauna Republicii Populare Române, Insecta, Fam. Nymphalidae*, 1965, **XI**, *7*, 3—361, 160 fig. XXV pl.
26. — Bull. de la Soc. Ent. de Mulhouse, 1967, sept-oct., 71—81.
27. — St. Cercet. Biol., Seria Zool., 1968, **20**, *3*, 215—225.
28. — Mitt. Ent. Ges. (Basel), 1968, 18, *1*, 27—31.
29. — Rev. Roum. Biol., Série Zool., 1969, **14**, *1*, 43—45.
30. — Linneana Belgica, 1970, Paris IV, *4*, 81—84.
31. — Bull. de la Soc. Ent. de Mulhouse, 1970, janvier-février, 1—16.
32. — St. Cercet. Biol., Seria Zool., 1970, **22**, *4*, 309—319.
33. NICULESCU E. V. et KÖNIG FR., *Fauna Republicii Socialiste România, Insecta, Lepidoptera, Partea generală*, 1970, **XI**, *10*, 303, 129 fig., XXIX pl.
34. POPESCU-GORJ A., Anal. R.P.R., 1951, **III**, Mem. 14; Bul. Şt. Acad. R.P.R. 1952, **IV**, 1; Trav. Mus. Hist. Nat. « Gr. Antipa », 1962, **III**; 1963, **IV**; 1965 **V**.
35. TUXEN S. L., *Taxonomist's glossary of genitalia in insects*, 1970, Ed. II, 359 p., Copenhague.
36. VERITY RUGERO, *Le Farfalle diurne d'Italia*, 1940—1953. I, II, III, IV, V, Marzocco, Firenze.
37. WARREN B. C. S., Trans. Roy. Ent. Soc. London, 1955, **107**, 381—392, pl. 1—4.

Reçu le 3 août 1971

THE EAST-ASIAN BARBINE MINNOWS WITH A PRECUMBENT PREDORSAL SPINE (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

The East-Asian Barbinae with a precumbent predorsal spine are ascribed to a distinct genus, *Spinibarbus* Oshima (= *Spinibarbichthys* Oshima), characterized by 5-branched anal rays (as against 6—8 in the South-Asian *Mystacoleucus*); it includes only 4 species. *Spinibarbus nigrodorsalis* and *Barbus caldwelli* are considered subspecies of *Spinibarbus hollandi*, *S. elongatus* a synonym of *S. hollandi*. *B. (S.) pingi* is a synonym and *S. denticulatus* a subspecies of *S. sinensis*. *Tor zonatus* Lin actually is a species of *Spinibarbus*. *S. hollandi* and *S. sinensis* are primitive, *S. macracanthus* and *S. zonatus* rather specialized species.

Several, rather dissimilar South-East and East-Asian species of Barbine Cyprinidae are characterized by the presence of a sharp precumbent spine in front of the dorsal fin. The species from Burma, Thailand, Indochina and Indonesia bearing this character are ascribed by most authors to a peculiar genus, *Mystacoleucus* Günther, 1868. There is a disagreement concerning the East-Asian species; Oshima [6] [7] proposed two distinct genera for them: *Spinibarbus* Oshima, 1919 (type: *S. hollandi* Oshima) for the species in which the last simple dorsal ray is slender and smooth, and *Spinibarbichthys* Oshima, 1926 (type: *S. denticulatus* Oshima, 1926, conspecific with *Barbus sinensis* Sauvage & Dabry, 1874) for those with spinified and serrated last dorsal simple ray. Lin [2] and Pellegrin & Chevey [8] recognize only *Spinibarbus*, including in it also the species with serrated dorsal spine; Rendahl [10] [11] considers both nominal genera as valid subgenera of *Mystacoleucus*, while Nichols [4]

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, Nº 2, p. 107—113, BUCAREST, 1972

[5] considers *Spinibarbus* a subgenus of *Barbus*, without recognizing *Spinibarbichthys* as distinct.

While the species from Burma, the Mekong and Menam drainages and Indonesia with predorsal spine have 6—8 or even more branched anal rays, those from East Asia (including the Song-Koi drainage in the Vietnam Democratic Republic and the coastwise rivers from Central Vietnam) have constantly 5-branched anal rays. The number of anal rays is a systematically very important character within the Cyprinidae; according to my knowledge, *Mystacoleucus*, *Rohtee*, *Cyprinion* and *Schizocypris* are the only genera of Barbinae with more than five-branched anal rays. The degree of ossification of the dorsal spine undergoes, on the contrary, strong variation within groups of closely related species (e.g. within the European *Barbus* s. str.). I consider therefore *Spinibarbus* as generically distinct from *Mystacoleucus*, and *Spinibarbichthys* a synonym of the first named.

MATERIAL

Some 150 specimens, including five holotypes, were examined; they belong to the following collections: Academy of Natural Sciences in Philadelphia (A.N.S.P.), American Museum of Natural History, New York (A.M.N.H.), British Museum, Natural History (B.M.N.H.), Field Museum of Natural History, Chicago (F.M.N.H.), Institutul de Biologie "Tr. Săvulescu", Bucharest (I.B.T.S.), Muséum National d'Histoire Naturelle, Paris (M.N.H.N.), Naturhistorisches Museum, Wien (N.M.W.), Rijksmuseum van Natuurlijke Historie, Leiden (R.M.N.H.), United States National Museum, Washington (U.S.N.M.), Zoologisches Institut und Museum, Hamburg (H.Z.S.).

SYSTEMATIC ACCOUNT

1. **Spinibarbus hollandi** Oshima, 1919. Figs 1, 2.

Synonym: *Spinibarbus elongatus* Oshima, 1920.

Subspecies: 1) *Barbus caldwelli* Nichols, 1925 (= *Mystacoleucus mandarinus* Rendahl, 1926); 2) *Spinibarbus nigrodorsalis* Oshima, 1926. Specimens examined:

— From Taiwan (Formosa) Island (*S. hollandi hollandi*):
Holotype of *S. hollandi*, F.M.N.H. 59095, Sobun R., 276 mm.
Holotype of *S. elongatus*, A.N.S.P. 49949, Buraku R., 181.6 mm. st. l.
A.N.S.P. 63172—63173, Kwaren R., 2 spec., 138.9 and 151.8 mm.
U.S.N.M. 191268, Hualien Hsien, 2 spec., 51.0 and 51.2 mm.
— From Hsikiang drainage, continental China (*S. hollandi hollandi*):
U.S.N.M. 94868, Poseh, Kwangsi Province, 1 spec., 95.2 mm.
A.N.S.P. 84974, same locality, 1 spec., 95.0 mm.
A.M.N.H. 12775, same locality, 1 spec., 87.5 mm.
R.M.N.H. 15338, same locality, 1 spec., 100.0 mm.

— From Minkiang R. drainage (Fukien Province) (*S. hollandi caldwelli*):
Holotype of *B. caldwelli*, A.M.N.H. 8434, Yenping, 100 mm.
A.M.N.H. 11050, Kienning, 11 sp., 61.0—219.0 mm.
A.M.N.H. 10223, 10716, 10235, all from Yenping (= Nanping), 12 specimens in all, up to 155.0 mm.
F.M.N.H. 14715 and 14716, same locality, 2 spec., 51.0 and 69.0 mm.
A.M.N.H. 11060, Chungan Hsien, 3 spec., 161.0—192.0 mm.
A.M.N.H. 10726, Fuching Hsien, 1 spec., 44.0 mm.
U.S.N.M. 130564, Foochow, 2 spec., 92.0 and 234.0 mm.
H.Z.S., no number, 4 spec., 65.5—117.2 mm.
Private collection, upper Minkiang R., 4 spec., 51.0—87.0 mm.
— From Hokou, Kiangsi, lower Yangtze (*S. hollandi caldwelli*):
A.M.N.H. 11071, 2 spec., 91.0 and 102.0 mm.
— From Hainan Island (*S. hollandi nigrodorsalis*):
A.M.N.H. 10698, 10719, 8367, 10757, all from Nodoa, 56 spec. in all, 31.5—177.0 mm.
N.M.W. 5993, Hainan, 1 spec., 85.0 mm. (labelled *B. hollandi*).
— From Song Koi drainage, Vietnam D.R. (*S. hollandi nigrodorsalis*):
M.N.H.N. 1937—15, "Tonkin", 1 spec., 277.0 mm.

The comparison of all these 109 specimens, as well as that of the description of *S. hollandi*, *S. elongatus*, *B. caldwelli*, *M. mandarinus* and *S. nigrodorsalis* convinced me that they belong to a single species, characterized by: last simple dorsal ray slender and smooth; lower profile almost horizontal, upper profile arched; mouth subterminal, horse-shoe shaped, lips thin, smooth, groove behind lower lip broadly interrupted in the middle; nine, rarely 8- or 10-branched rays in the dorsal; body darker above, paler below lateral line but without spots; distal edge of dorsal fin marked by a strong blackish stripe. The body proportions are about the same in all populations examined: body depth 22.4—28.0% of st. length, rarely 30.2% (but 20.4% in the holotype of *S. elongatus*); least depth 9.9—12.2% (9.7% in the holotype of *S. elongatus*); caudal peduncle length 14.7—18.4%; predorsal distance 46.2—53.0%; preventral distance 50—56% (rarely 48.6%); distance from pectoral to pelvic origin 24.8—31.4%; distance from pelvic to anal origin 20.8—27.8%; head length 26.4—31.2%; snout length 7.0—9.4% (rarely up to 10.6%); eye diameter 3.8—6.4%.

The most variable character is the number of scales in lateral line, which allows the recognition of three subspecies:

Spinibarbus hollandi hollandi with 25—29 scales (those on caudal fin base included); the mean values are: 27.5 ± 0.32 in the specimens from Taiwan, 26.6 in those from Kwangsi, Hsikiang drainage, which appear thus somewhat intermediate between the subspecies *hollandi* and *caldwelli*, being much closer to the first named.

Sn. elongatus Oshima (Fig. 2) is based on a single specimen with an exceptionally low body and caudal peduncle; it has 28—29 scales, as against 26—27 in the type of *hollandi* and 27—29 in the other available Taiwan specimens. The body depth being subject to a strong individual, partially allometrical variation, I cannot recognize *elongatus* as distinct species.

Spinibarbus hollandi caldwelli (Nichols) (= *M. mandarinus* Rend.), characterized by 23—26, rarely 27 scales; the mean value is 24.69 ± 0.13

in the Minkiang drainage specimens. In the two available specimens said to be from Hokou, Kiangsi, lower Yangtze, I found 25 scales in one specimen, 25—27 in the second (M = 26.6); yet I doubt these specimens are from the lower Yangtze, for they may have been caught in some tributary from the upper Minkiang or in some Chekiang river not far from Hokou; Nichols records from Hokou also other species otherwise known only from Fukien or Chekiang.

Nichols [5] recognizes *M. mandarinus* Rendahl, 1926 as specifically distinct, in spite of the fact that Rendahl himself synonymized later on this nominal species with *caldwelli* [10] [11].

Spinibarbus hollandi nigrodorsalis Oshima: scales in lateral line 21—25 (M = 23.6±0.19) in Nodoa island specimens, 24—25 in the only available North Vietnam specimen.

One remarks a sharp difference in the number of scales between *hollandi* on the one hand, *caldwelli* and *nigrodorsalis* on the other. Adopting the 75% principle, one would not be justified to accept *nigrodorsalis* as subspecifically distinct from *caldwelli*. Yet I accept both *nigrodorsalis* and *caldwelli* as valid subspecies, because their ranges are separated by the Hsikiang drainage, which is inhabited by populations very close and taxonomically identical with the nominal subspecies from Taiwan Island. The great similarity between the *S. hollandi* forms from Taiwan and the Hsikiang drainage, as against the dissimilarity between those from Taiwan and Fukien (which lie geographically so close) contrasts the situation in most other fresh-water fishes.

2 a. **Spinibarbus sinensis sinensis** (Sauvage & Dabry, 1874)

Synonym: *Barbus* (*Spinibarbus*) *pingi* Tchang, 1931

Specimens examined:

Holotype of *Barbus sinensis*, M.N.H.N. 50.27, "China", 200 mm. st. l.
M.N.H.N. 34—19, "China", 1 spec., 135.0 mm.
A.M.N.H. 15273, Omei, Szechwan, 1 spec., 166.0 mm.
U.S.N.M. 130112 & 87190, "China" (probably Szechwan), 2 specimens, 239.0 and 253.5mm.
F.M.N.H. 43611, Kiating, Szechwan, 1 spec., 189.0 mm.
F.M.N.H. 43829, "China" (probably Szechwan), 1 specimen, 189.0 mm.
F.M.N.H. 51117, Suifu, Szechwan, two spec., 142.0 and 143.0 mm.
B.M.N.H. 1969. 4.15: 111, Szechwan, one spec., 340.0 mm., labelled *S. pingi*.

D III 9—10; L. lat. $29\frac{6}{3-3\,1/2}33$ (34); Sp. br. 10—13; D. ph. 5.3.2—2.3.5.

Last simple dorsal ray ossified and serrated, but less than in *S. macracanthus* and *S. zonatus*. General habitus quite variable, in some specimens the lower profile is, as in *S. hollandi*, almost horizontal (Fig. 4), in other specimens it is almost as convex as the upper profile (Fig. 3). Body depth 28.8—35.6% of st. length; least depth 12.4—15.1%; caudal peduncle length 17.8—20.6%; predorsal distance 45.5—51.2%; preventral distance 46.0—51.8%; distance from pectoral to pelvic origin 26.0—32%; distance from pelvic to anal origin 25.0—30.6%; head length 21.2—25.2%; snout length 6.1—7.7%; eye diameter 4.1—6.4%. Pelvic origin always

behind vertical from dorsal origin, often under vertical from centre of dorsal base. Lips as in *S. hollandi*.

Darker above, paler below; scales on body sides, especially above lateral line, bordered with brownish; no spots on bode. Extremities of fins darkened, those of anal and pelvics almost blackish, but no black stripe on edge of dorsal, as in *S. hollandi*.

According to its original description by Tchang [12], *Barbus pingi* is a synonym of *S. sinensis*, as accepted also by Nichols [5].

Upper Yangtze drainage; not recorded from the lower Yangtze.

2 b. **Spinibarbus sinensis denticulatus** Oshima, 1926. Fig. 5.

Synonym: *Spinibarbus spinicelatus* Koller, 1927.

Specimens examined:

A.M.N.H. 11704, Hainan Isl., 2 specimens, 128.5—172.0 mm.
A.M.N.H. 1071, 11051, 10729 Nodoa, Hainan Island, 27 specimens in all, 51.0—178.0 mm.
R.M.N.H. 15323, Posch, Kwangsi (Hsikiang drainage), 1 sp., 135.0 mm.
U.S.N.M. 94858, Kwangsi, 1 spec., 160.0 mm.
M.N.H.N. 1937—16, Vietnam D.R. (Song Koi drainage), 1 spec., 169.0 mm.
N.M.W. 5077, Wu-Tschli Mts., Hainan, 1 spec., 157.0 mm. (labelled *Spinibarbus spinicelatus*, apparently one syntype of the last-named nominal species.

D III 9; L. lat. (28) $29\frac{5—5\,1/2}{2\,1/2—3}32$; Sp. br. 9—12.

Last simple dorsal ray spinified and serrated, as in *S. s. sinensis*. Agrees with *S. s. sinensis* in all characters, except the position of the pelvic fin which is opposite to that of the dorsal. Body proportions as in *S. s. sinensis*: depth 26.8—34.6% of st. length, least depth 11.1—13.3% etc. but predorsal distance 50.9—56.5% (as against 45.5—51.2%).

Hsikiang drainage in continental China; Hainan Island; Song Koi R. drainage in the Vietnam D.R.

3. **Spinibarbus macracanthus** Pellegrin & Chevey, 1936.

Specimens examined:

Holotype of *S. macracanthus*, M.N.H.N. 36.2, Central Vietnam (south Anam), 283.5 mm. (Fig. 6).
M.N.H.N. 36.3, same locality, 1 specimen, 71.0 mm.
I.B.T.S. 0633, Boi R., Vietnam D.R., 1 spec., 94.0 mm.

D III 9; L. lat. $36\frac{8}{3-4}40$; Sp. br. 13—14; D. phar. 5.3.2—2.3.5.

Depth 28.8—31.6% of st. length; caudal peduncle 20.0—21.0%; least depth 11.1—11.7%; predorsal distance 49.0—56.0%; preventral distance 50.0—51.0%; distance from pectoral to pelvic origin 22.3—24.0%; from pelvic to anal 21.4—25.5; head 25.5—26.7%; snout 8.4—10.6;

eye 5.6—8.4%. Lower profile slightly convex, upper profile rather strongly arched. Dorsal fin deeply notched, its last undivided ray much stronger than in *S. sinensis*, spinified and serrated distally. Mouth inferior (as against subterminal in *S. hollandi* and *S. sinensis*); lower lip continuous, its median part broad, somewhat expanded, with slight longitudinal furrows, delimited posteriorly by a groove (Fig. 7).

Some five faint vertical stripes on body sides above lateral line, not reaching to back; scales above lateral line bordered with brown. Fins colourless.

Song Koi R. drainage and coastwise rivers from the Vietnam Democratic Republic, may be from the eastern part of South Vietnam too.

4. **Spinibarbus zonatus** (Lin, 1935)

Synonym: *Tor zonatus* Lin, 1935; *Barbus* (s.str.) *zonatus*, Nichols, 1943.

Specimens examined:

U.S.N.M. 94866, Linchow, Kwangsi (Hsikiang drainage), leg. S. Y. Lin, 1 spec., 148.2 mm (Fig. 8, 9).

A.N.S.P. 101345, same locality and collector, 1 spec., 113.4 mm.

D III 9—10; L. lat. 42 $\frac{8}{5}$ 45; Sp. br. 23—26; D. phar. 4.3.1 — 2.3.5; 5.3.2—2.3.5.

Body depth 25.2—27.6% of st. length; caudal peduncle 19.4—23.6%; least depth 9.4—10.2%; predorsal distance 47—48%; preventral distance 47.0—50.5%; distance from pectoral to pelvic origin 23—24%; from pelvic to anal 24—25%; head 26.4—27.4%; snout 8.7—10.2%; eye diameter 6.7—7.2%. Lower profile almost horizontal, upper profile arched, with an elevation at dorsal base. Dorsal fin deeply notched, its spine strong, almost smooth proximally, serrated distally. Mouth inferior; lower lobe continuous, with a well developed symphyseal lobe, reaching as far posteriorly as the lateral lobes (Fig. 8); the shape of the lip represents a reverse 3. Two pairs of barbels (as in the other species), the lacrimal pair sometimes hidden under the lacrimal.

Five or six large but faint transverse stripes, reaching from back almost to the ventral side.

Restricted to Hsikiang drainage.

Lin [3] has overlooked the precumbent predorsal spine, which is as developed as in the other species of the genus; he ascribes therefore *zonatus* to *Tor*. Actually it is a *Spinibarbus*.

★

The four species here included in *Spinibarbus* differ sharply in general habitus, degree of development and ossification of the last unbranched dorsal ray and shape of the lower lip. Yet I think they actually are related. *S. hollandi* seems to represent the more primitive condition; *S. sinensis* is rather close to *hollandi*, differing from it mainly in the ossified and serrated dorsal spine, while *S. macracanthus* and *S. zonatus*, each of them with a peculiar shape of lower lip, are the most specialized species within the genus.

Acknowledgements. Following curators lent specimens under their care or facilitated their study: M-me le Prof. M.-L. Bauchot, Paris; Dr. M. Boeseman, Leiden; Dr. J. Böhlke, Philadelphia; Dr. P. H. Greenwood, London; Dr. P. Kähsbauer, Wien; Dr. E. A. Lachner, Washington; Dr. W. Ladiges, Hamburg; Dr. D. E. Rosen, New York; Dr. L. P. Woods, Chicago. A visit to several museums in the U.S.A. and to the British Museum was financed by the Smithsonian Institution's T.F.H. Fund, thanks to the kind recommendation of Dr. E. A. Lachner.

REFERENCES

1. Koller, O., Ann. Naturhistor. Mus. Wien, 1927, **41**, 25—49.
2. Lin S. Y., Lingnan Sci. J., 1933, **12**, *2*, 197—215.
3. — Lingnan Sci. J., 1935, **14**, 303—313.
4. Nichols J. T., Amer. Mus. Novit., 1925, **185**, 1—7.
5. — *The Fresh-water Fishes of China.* The American Museum of Natural History, New York, 1943.
6. Oshima M., Annals Carnegie Mus., 1919, **12**, *2—4*, 169—328.
7. — Annot. zool. Japon, 1926. **11**, 1—26.
8. Pellegrin J., Chevey P., Bull. Soc. zool. France, 1936, **61**, 375—379.
9. Rendahl H., Ark. f. Zool., 1926, **18 B**, *11*, 1—4.
10. — Ark. f. Zool., 1928, **20 A**, *1*, 1—194.
11. — Ark. f. Zool., 1932, **24 A**, *16*, 1—134.
12. Tchang Tch. L., Bull. Fan Memor. Inst. Biol., 1931, **2**, 225—242.

Received October 12, 1971

The "Traian Săvulescu" Institute of Biology
Department of Systematics and Evolution of Animals

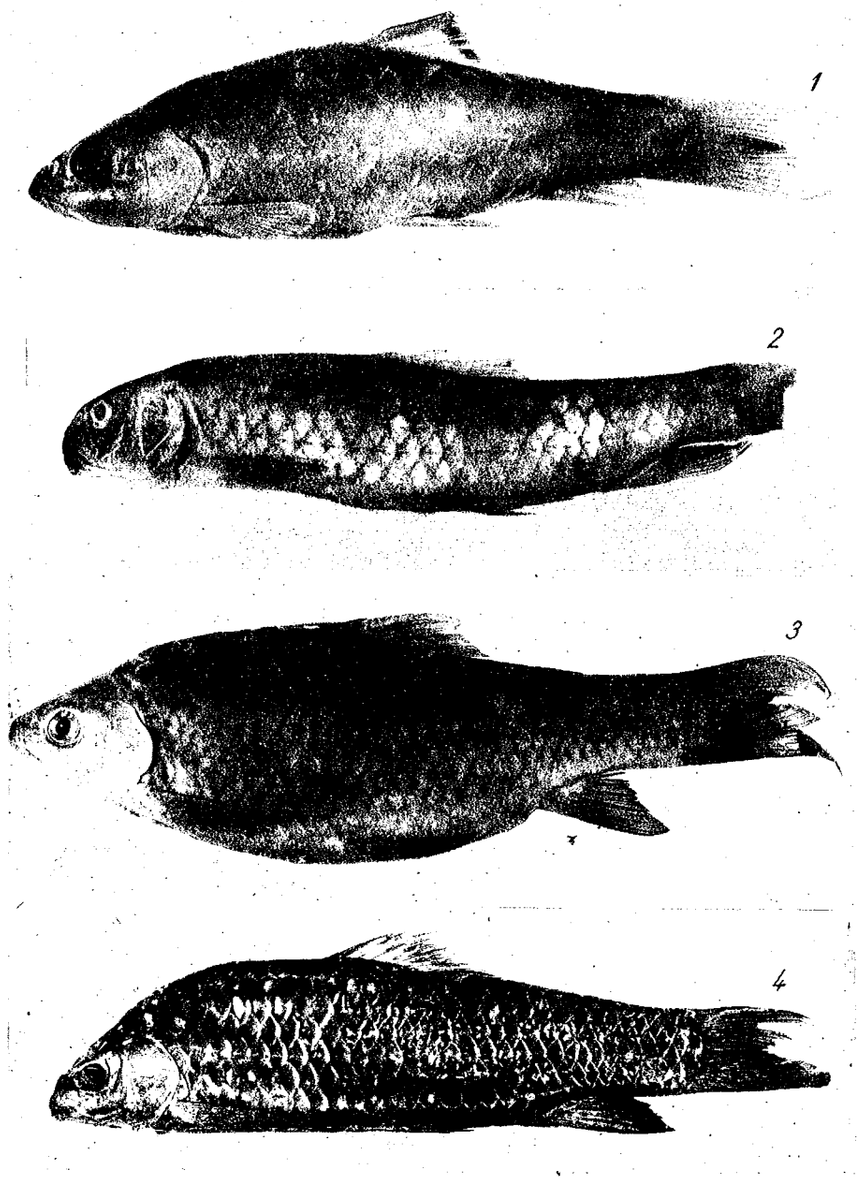


Fig. 1. — *Spinibarbus hollandi caldwelli* (Nichols). Upper Minkiang R., Fukien ; leg. Dr. Wu H. W.
Fig. 2. — *Spinibarbus hollandi hollandi* Oshima. Holotype of *S. elongatus* Oshima, A.N.S.P. 49949 ; courtesy of Dr. J. Böhlke.
Fig. 3. — *Spinibarbus sinensis sinensis* (Sauvage & Dabry). F.M.N.H. 43611 ; Kiating, Szechwan.
Fig. 4. — *Spinibarbus sinensis sinensis* (Sauvage & Dabry). B.M.N.H. 1969.4.15 : 111 ; Szechwan (labelled *S. pingi*).

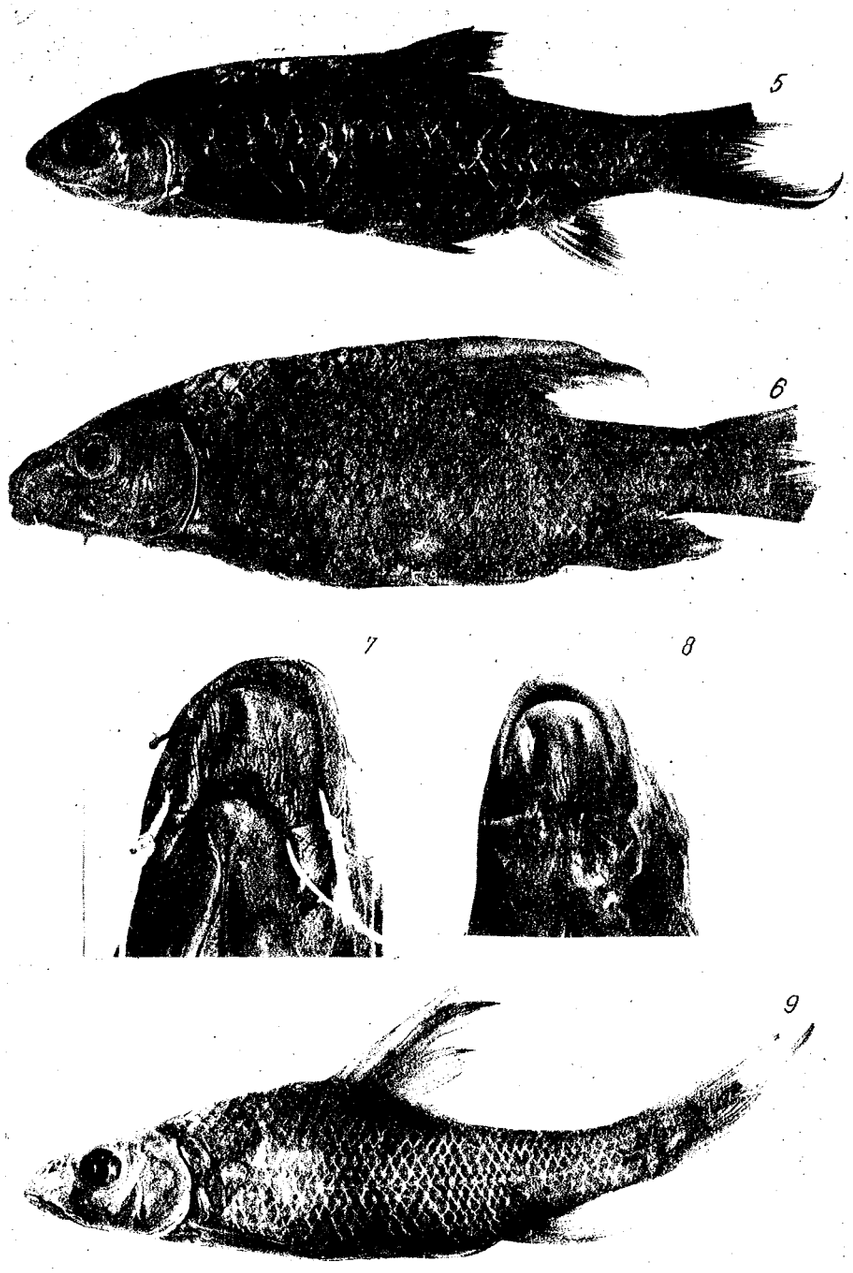


Fig. 5. — *Spinibarbus sinensis denticulatus* (Oshima); syntype of *S. spiniceladus* Koller; N.M.W. 5077, Wu-Tschil Mts., Hainan.
Fig. 6. — *Spinibarbus macracanthus* Pellegrin & Chevey, holotype; M.N.H.N. 36–2; Central Vietnam.
Fig. 7. — *Spinibarbus macracanthus*, ventral view of mouth.
Fig. 8. — *Spinibarbus zonatus*, ventral view of mouth.
Fig. 9. — *Spinibarbus zonatus* (Lin); U.S.N.M. 94866; Linchow, Kwangsi.

ON THE DEVELOPMENT OF THE CHONDROCRANIUM IN *SALMO TRUTTA FARIO* AND *LEUCASPIUS DELINEATUS*

BY

G. T. DORNESCU and CONSTANTINA SORESCU

The development of the skull in bony fishes has been the concern of anatomists since long. Parker (1873) was the first to study the development of *Salmo*, followed by Gaupp (1906), Böker (1913), Pehrson (1922), Beer (1927), Goodrich (1930), Tchernavin (1937), Holmgren and Stensiö (1940), Hubendick (1942) and Bhargava (1958). The development of the chondrocranium in *Polypterus*, *Amia* and *Lepidostens*, *Acipenser*, *Salmo* and *Leuciscus rutilus* (= *Rutilus rutilus*) is well known but previous investigations were devoted only to chondrocranium formation. The formation of the osteocranium in bony fishes and the presence of the polar cartilage are still unknown problems. The origin of cranial bones in *Teleostei* was determined using data obtained after studying the skull of mammals, a group of evolved vertebrates where membrane and enchondral bones are separated phylogenetically.

The comparative study of the chondrocranium development in *Salmo trutta fario* and *Leucaspius delineatus* in the present work is aimed at elucidating three questions concerning the Teleostean skull: the existence of the polar cartilage, the role of trabeculae in determining the type of tropibasic skull and the origin of cranial bones.

MATERIAL AND METHOD

The development of the chondrocranium was followed up on serial sections, in larvae and alevins of the two genera, of various lengths (5 mm., 6 mm., 10 mm., 16 mm., 21 mm.). The studied material was fixed in Bouin and Bouin-Hollande fluid, embedded in paraffin, sectioned at 6 μ and 8 μ and stained with hemalum-erythrosin.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, No 2, p. 115–119, BUCAREST, 1972

PERSONAL INVESTIGATIONS

Before eclosion, in the membranous primordial cranium there appear trabeculae, parachords and auditory capsules.

In *Salmo trutta fario*, *Leucaspius delineatus* and *Cyprinus carpio* [5], development lines are the same though the chondrocardium develops slower in *Salmo* than in *Leucaspius* and *Cyprinus*. Thus, in all Teleostei studied, the cartilaginous base of the chondrocranium is already formed at eclosion. Each side of the notochord presents a parachordal cartilage which is caudally joined to the notochord, and rostrally continues directly with *trabecula cranii* without individualizing a polar cartilage, similar to that of Chondrichtyes.

Each *parachordalia cranii* is laterally fused to the auditory capsule by the anterior basicapsular commissure [5].

In alevins of *Salmo trutta fario* (16 mm. long), *Leucaspius delineatus* (10 mm. long) and *Cyprinus carpio* (7 mm. long), all cranial cartilages have grown and the chondrocranium floor has become a basal plate.

This plate is formed medially and ventrally of *trabeculae cranii* and *parachordalia cranii*, latero-anteriorly of *taenia marginalis* (= orbital cartilage), and latero-posteriorly of the postorbital process (= lateral commissure), anterior basicapsular commissure and posterior basicapsular commissure [5].

If skull areas are studied in cross section, starting with the ethmoid region, it may be noticed that *trabeculae cranii* are anteriorly fused forming the *trabecula communis*. This is strongly widened rostrally in an ethmoid plate or *solum nasi* (Figs 1 and 2), which extends posterolaterally in *lamina orbitonasalis* and forms medially a vertical cartilaginous lamella, called *septum nasi* (Fig. 1).

Taenia marginalis in this area divided into two cartilages : a horizontal one (the sphenoseptal commissure) uniting with *septum nasi* (Fig. 3), and a vertical one (sphenoethmoidal commissure), joining the *lamina orbitonasalis*, on the same side (Fig. 5).

The two commissures, *lamina orbitonasalis* and the ethmoidal plate delineate the olfactory opening (Fig. 5), forming part of the side wall of the neurocranium, in the ethmoid region and in the rostral part of the orbital region.

As the section is carried out at eye level, it is observed that *trabecula communis* is still maintained, yet much more reduced, not being involved in the closure of the cranial floor, which is made up only of the connective tissue, to form the desmal bone.

In the caudal part of the orbital region, each *teania marginalis* appears under the form of a cartilaginous chord, placed dorso-laterally to the encephalon, while the ethmoidal plate continues with two lateral lamella.

Trabecula communis decreases more (Figs 6 and 7) near the auditory region, where all described cartilages disappear and are replaced ventrally by *parachordalia cranii* and latero-ventrally by auditory capsules (Fig. 8).

Parachordalia cranii spread along the dorsal chord, being connected to auditory capsules by two cartilaginous commissures : the anterior basicapsular commissure (Fig. 9) and the posterior basicapsular commissure (Fig. 10). Between *parachordalia cranii* and the anterior basicapsular commissure there is the anterior basicapsular fenestra. *Parachordalia cranii* and the posterior basicapsular commissure delineates the posterior basicapsular fenestra. The side wall of each auditory capsule increases dorsally and shelters the auditory sacs (Fig. 9). Posteriorly, on these walls a septum of the posterior semi-circular canal is formed, separating the canal from the remaining auditory cavity (Fig. 10).

The caudal wall of the auditory capsules is fused to occipital arcs. Between the formed cartilages, remnants of the membranous primordial cranium are maintained.

In this way, cartilaginous patterns of the enchondral portions from the mixed bones were outlined.

DISCUSSIONS

The presence of the polar cartilage in Teleostei is a much discussed problem. In cartilaginous fishes, it is obvious that between trabeculae and parachords there is a pair of polar cartilages, well characterized, making the connection between the two primary cartilages [8]. In *Salmo*, *Leucaspius* and *Cyprinus carpio*, no formation comparable to this polar cartilage was observed. Trabeculae and parachords are directly fused immediately after their formation, even before eclosion.

It is possible that polar cartilages may be fused to parachords, making up their anterior end [1], in Teleostei. It may be asserted with certainty that these cartilages do not appear as separate chondrifications. Some researchers considered as polar cartilages [8] formations which probably represent peculiar forms of the parachords. It is obvious that, though the general development lines of the chondrocranium are the same in all Teleostei, there are variations in the form of primary cartilages (trabeculae and parachords), specific of every taxon (Figs 1—6). These variations are maintained also in the skull of adults and some may be used as phylogenetic indices. The question of the tropibasic and platybasic skull is correlated to the differentiation of osteologic characters, with a view to using some of them in phylogeny.

Modern systematics indulges ever more in the idea of using the skull type for creating phylogenetic schemes. We consider that this cranial characteristic has no value in phylogeny, since it is directly submitted to the influence of easily modified external factors.

Thus, the type of tropibasic skull (with interorbital septum and close eyes) is achieved by bringing near trabeculae and fusing them together in a *trabecula communis* (dermic interorbital septum is supported by this trabecula). In its turn, the existence of the *trabecula communis* depends on the relative size of the eyeballs and of the encephalon. This is confirmed by the possibility to obtain experimentally a platybasic cranium instead of the tropibasic one, characteristic of the species. This is obtained by treating the embryo with agents which produce cyclopia. Agents may be modified, influencing the size of eyes, which accounts for the appearance of tropibasia in species which are phylogenetically distanced.

Another interesting problem is to establish the origin of cranial bones in Teleostei. It has been recorded that in Teleostei some dermal portions could interpenetrate endochondrial ones in cranial bones [8], yet no studies could supply a satisfactory answer. Classical delimitations have been used, viz. membrane and cartilage bones, as the componence and origin of cranial bones are not known in this group of lower vertebrates.

The comparative study of the chondrocranium and osteocranium in *Salmo trutta fario* and *Leucaspius delineatus* as well as in *Cyprinus carpio* [5], [6] has pointed out that the skull of Teleostei has only two kinds of bones: desmal and mixed ones. The endochondrial portions of the mixed cranial bones originate in the following cartilages: *solum nasi* and *septum nasi* (for the ethmoid, figs 1 and 2); pleurethmoid (= lateral ethmoid) originates: dorsally in the sphenoseptal commissure and in the *taenia marginalis* (Figs 3 and 4), laterally in the sphenoethmoidal commissure and in the *lamina orbitonasalis* (Fig. 5) and ventrally in the lateral ethmoidall plate and in the *trabecula communis*; enchondral portions of the orbitosphenoid and pleurosphenoid bone derive from the *taenia marginalis* (Figs 6 and 7); those of auditory bones from the auditory capsule and parachords (Figs. 8, 9 and 10) and those of occipital bones from the auditory capsule, parachords and occipital arcs.

Between these primary cartilages of the chondrocranium there occur remnants of the membranous primordial cranium, which by direct ossification will form portions of the dermal bone of mixed bones, completing the neurocranium. In this way, cranial bones of the Teleostei will be mixed (except the desmal ones of the ceiling and floor, e.g. the frontal, parietal and parasphenoid ones). This permits us to assert that in Teleostei there is no squamosal bone. In its place, there is a desmal portion of the pterotic accurately called dermopterotic (Tilak, 1967). The dermopterotic is for the pterotic what is the latero-rostral process for the sphenotic bone and the wing for the occipital bone, viz. a membrane bone closely fused to the enchondral bone.

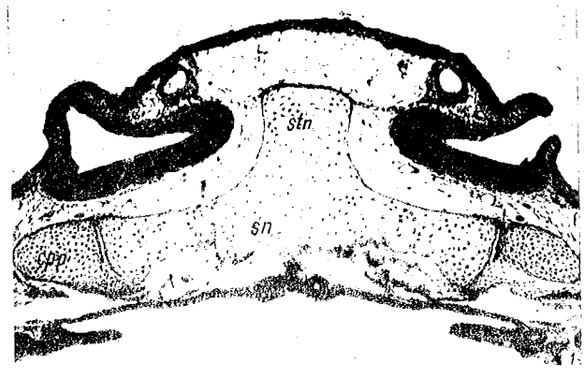
CONCLUSIONS

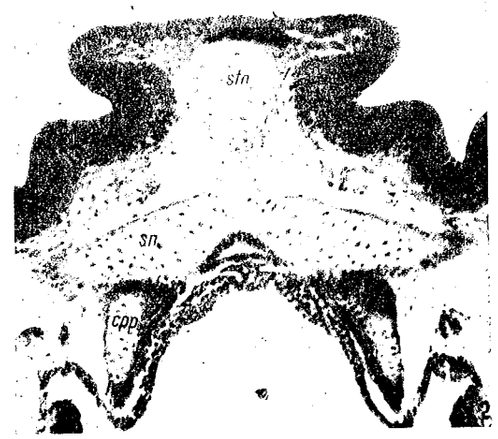
1. The chondrocranium of Teleostei is characterized by the absence of the polar cartilage (as a separate formation), by the presence of the *trabecula communis* which determines cranium tropibasia and by variations in the form of primary cartilages (trabeculae and parachords), characteristic of every taxon.

2. The presence of mixed bones in the skull, where membranous portions interpenetrate enchondral ones, permits us to consider that the evolution of cranium ossification in Teleostei occurs in the dermic-perichondral-enchondral sense, inversely than in vertebrates, where it is enchondral-dermic. This is another argument in favour of the theory of cranium segmentation.

3. In Teleostei there is no squamosal bone, and the pterotic is a mixed bone whose dorsal portion is made up of a dermopterotic.

4. The mixed componence of cranial bones in Teleostei is accounted for by the fact that between cartilages, the chondrocranium wall is made





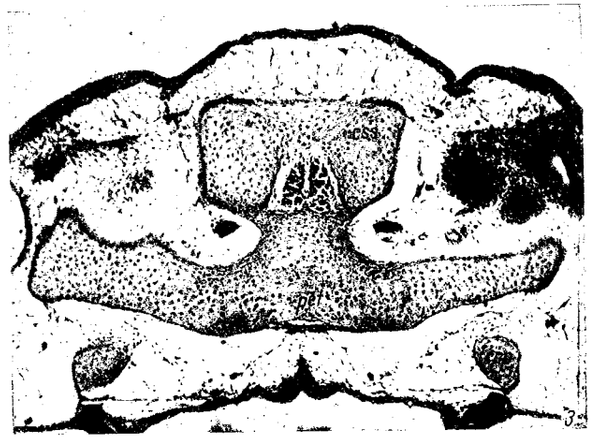
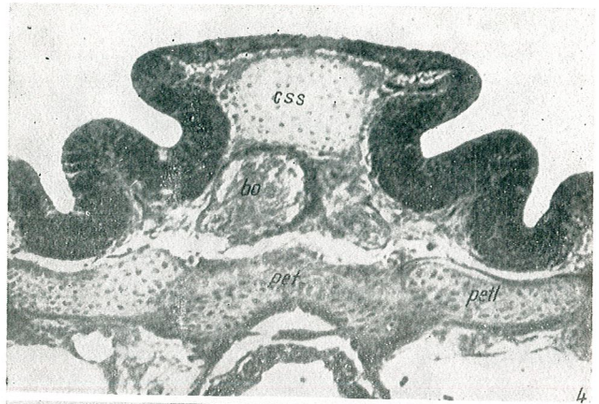


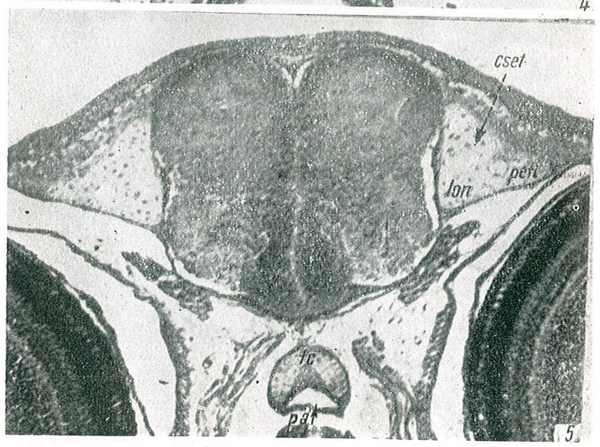
Fig. 1. — Cross section in the chondrocranium of a 16-mm. long alevin of *Salmo trutta fario*.

Fig. 2. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

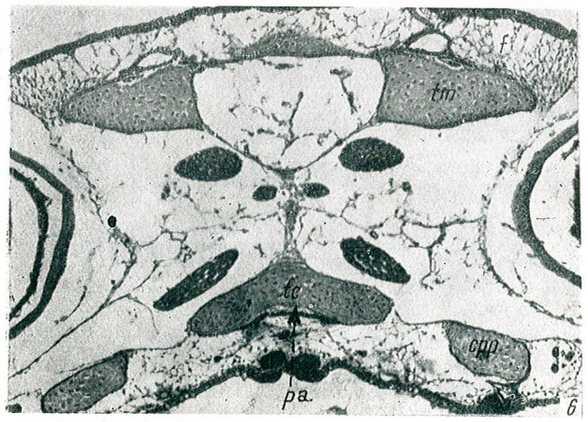
Fig. 3. — Cross section in the chondrocranium of a 16-mm. long alevin of *Salmo trutta fario*.



4



5

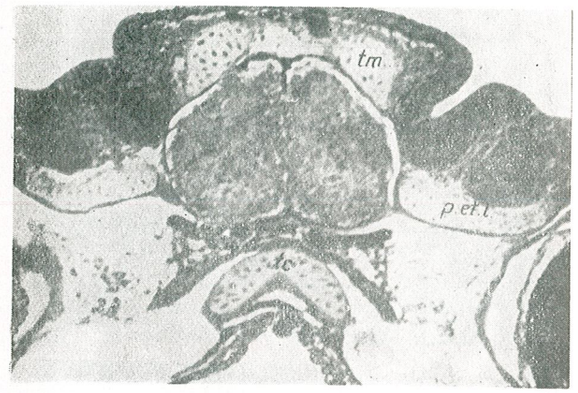


6

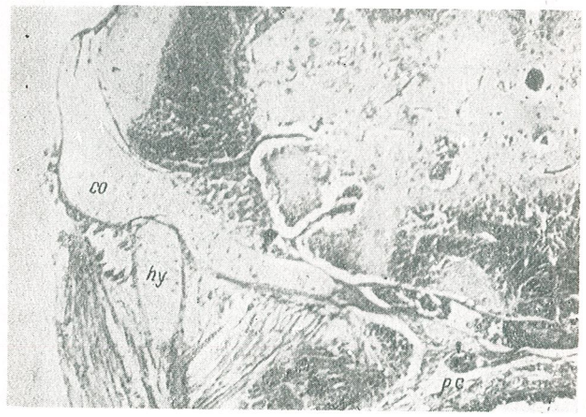
Fig. 4. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

Fig. 5. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

Fig. 6. — Cross section in the chondrocranium of a 16-mm. long alevin of *Salmo trutta fario*.



7



8

Fig. 7. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

Fig. 8. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

BIBLIOTECA
Inst. de Științe biologice-Buc.

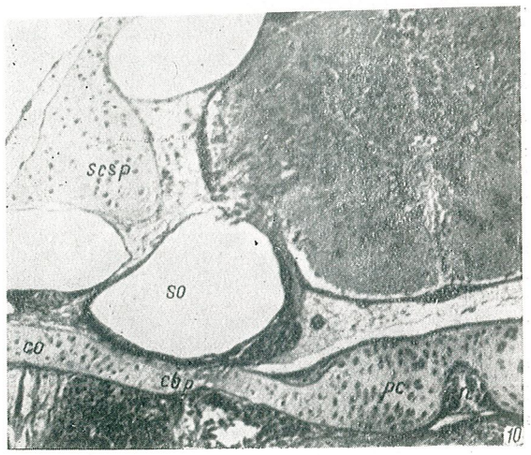


Fig. 9. — Cross section in the chondrocranium of a 16-mm. long alevin of *Salmo trutta fario*.

Fig. 10. — Cross section in the chondrocranium of a 10-mm. long alevin of *Leucaspius delineatus*.

up of remnants of the primitive meninx, which by direct ossification form both desmal bones and desmal portions of mixed bones.

REFERENCES

1. De Beer C. R., *The development of the Vertebrate skull*, Clarendon Press, Oxford, 1937.
2. Beccari N., *Anatomia comparata dei Vertebrati*, II, Firenze, 1955.
3. Bhargava N. H., Journ. Morph., 1958, **102**, *3*, 401.
4. Devillers Ch., in Grassé P. P., *Zoologie*, II, Masson, Paris, 1965.
5. Dornescu G. T., Dornescu M., Marcu H., Sorescu C., Anat. Anz., 1969, **125**, 296—302.
6. Dornescu G. T., Sorescu C., Anat. Anz., 1971, **128**, 16—38.
7. Goodrich E. S., *Studies on the structure and development of Vertebrates*, Macmillan, London, 1930.
8. Grassé P. P., *Traité de Zoologie*, 1954, vol. 12; 1958, vol. 13, f. *1*.
9. Sagemehl M., Morph. Jahrb., 1891, **17**, 489.
10. Swinnerton H. H., J. Micr. Sc., 1901—1902, **45**, 503.

University of Bucharest
Faculty of Biology
and
University of Craiova

Received November 13, 1971

ABBREVIATIONS

abc, anterior basicapsular commissure; *ac*, auditory capsule; *as*, auditory sac; *etp*, ethmoidal plate; *f*, frontal; *hy*, hyomandibular; *letp*, lateral ethmoidal plate; *lon*, lamina orbitonasalis; *n*, notochord; *ob*, olfactory bulb; *pa*, parasphenoid; *pbc*, posterior basicapsular commissure; *pc*, parachordalia cranii; *psc*, palatal square cartilage; *setc*, sphenoethmoidal commissure; *sl*, solum nasi; *spesc*, septum of the posterior semicircular canal; *ssc*, sphenoseptal commissure; *stn*, septum nasi; *tc*, trabecula communis; *tm*, taenia marginalis.

CLEAVAGE OF TUBIFEX EGGS UNDER VARIOUS CONDITIONS OF MAGNETIC FIELD APPLIED AT DIFFERENT PERIODS OF THE CELL CYCLE

BY

RODERICH BRANDSCH

The course of the first mitotic divisions of cleaving Tubifex eggs under different conditions of MF was investigated. It is probable that the gradient of the MF is responsible for the observed effect. Under MF the formation of the division spindle is inhibited, the cytokinesis takes place under very intense "bubbling" of the egg cortex, the cell division may be completely blocked. The aspect of the cleavage under MF is similar to that observed in the case of other inhibitors of cell division and is discussed in this connection.

During the last years more and more reports about the action of MF (magnetic field) have been accumulating. They often are, however, contradictory. Classifying the possible ways of interaction between MF and biological systems, Barnothy [2] indicates the following modalities: a deviation force displayed on the paths of charged particles and a torsion force which would act on permanent magnetic dipoles and on non-spherical para- and diamagnetic particles. The kind of force exhibited differs in the case of a homogeneous or inhomogeneous field. If the effect is a function of the square of the magnetic field intensity (H^2) or a function of the paramagnetic intensity of the field (HdH/dx), we have to correlate the effect with a paramagnetic phenomenon. If there exists a linear correlation between the observed effect and the field intensity or the field gradient, we have to consider an interaction of the field with some individual elementary magnets within the biological system. In the case of a great anisotropy of the molecular dipoles we could expect differences in the action of the magnetic field in correlation with the N—S polarity.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, Nº 2, p. 121–130, BUCAREST, 1972

Temperature differences should also influence the effect of a magnetic field, if we consider that, according to Valentinuzzi [1], the rotational diffusion of molecules is affected by the magnetic field, leading to an alteration of the reaction rates.

In respect to cell division several researches have been performed concerning the influence of homogeneous and inhomogeneous fields of different intensities on a variety of processes such as wound healing [1], cell cultures [14] [18], cleavage of Echinodermata eggs [2] [13] and cell division in root tips [6]. In general it seems that inhomogeneous fields show a greater influence than homogeneous ones and that there exists a linear correlation between the observed effect and field intensity and/or gradient.

But these works deal only with the problem whether there can be established an influence of the magnetic field used on cell division. It would be, however, of interest to determine more exactly which parameters of the MF are responsible for this action and also to follow up the dynamics of MF action during the cell cycle, in order to see whether there exist some periods particularly sensitive to MF. In this way it would become possible to correlate the action of the magnetic field with some determined processes.

As experimental model we used the first cleavages of the Tubifex egg. The course of division was examined under conditions of a homogeneous or inhomogeneous field of different intensities and gradients, at different temperatures and N—S orientations. We also tried to establish the action of the magnetic field during interphase, mitotic stages and cytokinesis.

MATERIAL AND METHODS

For the generation of MF two electromagnets were used. One was designed with some modifications after Levengood [12] and produced a strongly inhomogeneous, localized magnetic field. Around a 6 mm. thick and 95 mm. long iron rod of low remnant field, 55,000 turns of 0.08 copper wire were wound. One end of the rod was sharpened down to a tip of 0.1 diameter. At a supply of 12 mA the field intensity at the tip was approximately 1,600 Gauss. Reducing the current supply from 12 mA by steps of two mA, we obtained 6 intensities of approximately 16,000, 13,000, 10,500, 8,000, 5,000 and 2,000 Gauss. At the rod tip a very strong inhomogeneous field was created with a gradient of approximately 8k Gauss within the first 0.5 mm. and with a marked drop at 2,000 Gauss. The coils were powered by a 110 V accumulator or through a stabilizer.

The coil is mounted in a vertical position on a wooden support. Below the coil there is a movable box (Fig. 1). The eggs are introduced into the box within a glass tube of 3 mm. internal diameter, filled with physiological solution and into which the tip of the iron rod is penetrating. The box is also provided with a thermometer and a 4.5 V bulb for observations. As after a prolonged use at 12 mA the coil exhibits a rather small heating effect, between the coil and the box a tube was placed through which a water flow was passing, so as to prevent the temperature to exceed 25°C. With the egg at the bottom of the tube, the tip is carefully screwed down until it stands exactly over the egg, then the power supply is switched on.

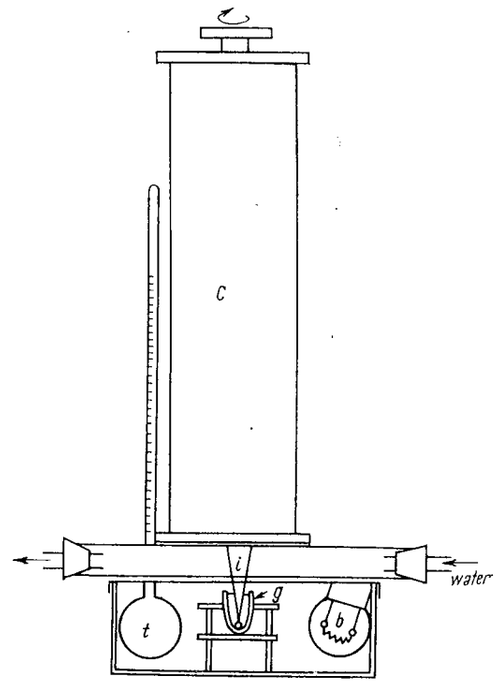


Fig. 1. — Electromagnet no. 1 : *c*, coil; *i*, iron rod; *g*, glass tube containing physiological solution and egg; *t*, thermometer; *b*, bulb. See text for detailed explanation.

The second electromagnet (Fig. 2) was designed to permit the generation of a homogeneous and an inhomogeneous magnetic field. Two coils of 0.08 mm. copper wire and 55,000 turns each were wound around a U-shaped iron rod of 6 mm. in diameter. At the free ends of the U-shaped rod two pole shoes were screwed. In order to produce a homogeneous field two cylindrical pole shoes with a diameter of 4 mm. at the top were used. At a pole gap of two mm. and a supply of 26 mA, the field intensity measured by means of a recorder based on Hall effect, was 1,300 Gauss. Extrapolating the values obtained for 2, 2.5 and 3 mm., the intensity of the MF at a gap of 0.5 mm. and a supply of 26 mA was approximated at 2,600 Gauss, whereas at a gap of 0.5 mm. and a supply of 50 mA, at 5,200 Gauss.

By exchanging one cylindrical pole with a pole having a tip of 0.1 mm. a strongly inhomogeneous field was created. The measured intensity at a gap of 2 mm. and a supply of 26 mA was 910 Gauss. Through extrapolation we obtained for 0.5 mm. and 26 mA an intensity of 1,600 Gauss and for 0.5 mm. and 50 mA an intensity of 3,600 Gauss.

Maintaining the supply at the same value and modifying only the gap between the poles from 2 mm. to 1 mm. and 0.5 mm. we obtained 3 different gradients of growing size. As it was not possible to measure the intensities at the pole surfaces at such distances, we cannot indicate numerical values for the gradients.

At 26 mA the temperature between the poles reached a maximum of 28°C, at which the divisions proceed normally. By introducing water through the double walls of the box it was possible to reduce the temperature to 18°C, which gave us the opportunity to watch the action of the MF with temperature. At 50 mA the coils were cooled continuously. The eggs were placed in physiological solution directly between the poles.

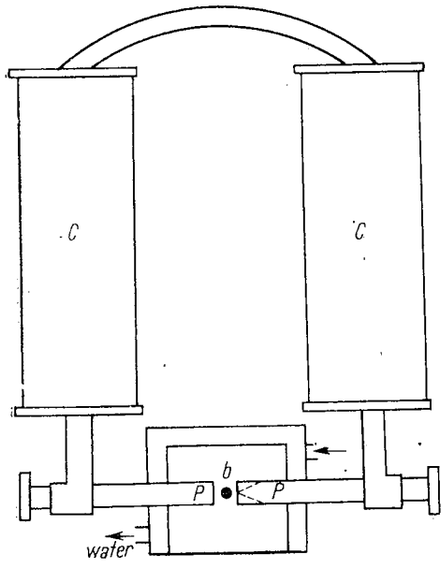


Fig. 2. — Electromagnet no. 2 : *c*, coil ; *b*, box containing physiological solution and egg, water-cooled ; *p*, exchangeable pole shoes. See text for detailed explanation.

As controls we used eggs under the same conditions but without power supply.

The eggs were exposed to the MF after the first or second reductional division, so that after a 5 hours treatment they stood before the first, respectively second mitotic division. To follow up the action of the MF along interphases, mitotic stages and cytokinesis, the period between the first reductional division and the second mitotic division was subdivided into periods of 60—90 minutes of treatment each.

Paraffin sections were effected at different times after treatment. Eggs were fixed with fixative E after Lehmann [10], sectioned at 5 u and stained with a mixture of acid fuchsin and light green.

RESULTS

On exposure to MF, the division proceeds under very strong "bubbling" of the egg cortex, screening the real furrow and leading even to the separation of some protoplastic fragments. Tubifex eggs being a typical example of spiraled segmentation, the furrows of the first divisions are separating cells of very different size. After exposure to MF the formation, at the second division, of two equal-sized C and D cells is characteristic, when there is no total block of cells division. Approximately 15% of all eggs treated with MF during 5 hours show a block of the first or second cell division. Eggs which underwent the first cell division may be stopped at the second one. It seems in general that the second mitotic division is easier to be stopped than the first one. Eggs which after a 5 hours treatment with MF do not achieve the next cell division may recover and carry on a few divisions, or they may remain blocked. Such undivided eggs live for a few days, from time to time exhibiting a new initiation of cell division.

At the beginning we tried to establish whether it is possible to correlate the appearance of the cleavage modifications described with certain parameters of the MF. As the ratio between blocked divisions and divisions which lead to the formation of equal C and D cells does not change, they were checked together as abnormal divisions. At first we compared the action of a homogeneous and an inhomogeneous field as concerns the appearance of abnormal divisions, expressed in percentage of total eggs treated, by means of electromagnet 2. The result is shown in table 1. It can be seen that in a homogeneous field, irrespective of

Table 1

Comparison between the action of a homogeneous and an inhomogeneous magnetic field

Homogeneous				Inhomogeneous		
intens.	divisions		intens.	divisions		
	nor.	abnor.		nor.	abnor.	block
control	24	2	control	24	2	0
1,300 G	30	3	900 G	34	5	0
dif.	unsig.		dif.	unsig.		
control	24	2	control	24	2	0
2,600 G	33	1	1,820 G	4	18	0
dif.	unsig.		dif.	$\chi^2 = 27$ $p<0.01$		
control	24	2	control	24	2	0
5,200 G	28	2	3,600 G	4	12	4
dif.	unsig.		dif.	$\chi^2 = 26.6$ $p<0.01$		

the increasing intensity, the percentage of abnormal cleavages in treated eggs does not differ from control. The situation is different in the case of the inhomogeneous field. The passing from 910 to 1,820 Gauss produces a very significant rise of abnormal divisions ($p < 0.001$). The percentage, however, does not increase when raising the intensity from 1,820 to 3,600 Gauss.

To produce an inhomogeneous field of a greater range of intensities we used coil no. 1. Figure 3 shows the relation between intensity and per-

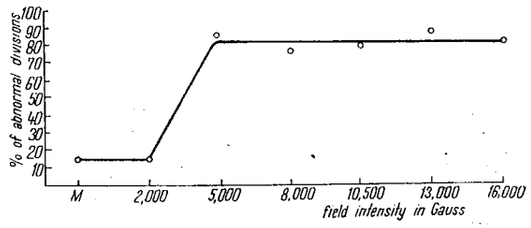


Fig. 3. — Variation of the percentage of abnormal divisions with the field intensity in the case of electromagnet no. 1. M, control.

centage of abnormal divisions. There is a statistically significant rise between 2,000 and 5,000 Gauss, after which the number of abnormal divisions remains approximately at the same level.

Thus it seems, at least under the conditions and with the material used, that only an inhomogeneous field affects cleavage and that this action does not depend on field intensity.

In order to see to what extent the effect, if not depending on intensity, may depend on the field gradient, we first compared the action of the MF on eggs put in the following experimental situation : two eggs were placed one on top of the other and were simultaneously treated in coil no. 1. Thus the upper cell near the electromagnet tip was exposed to a greater gradient than the lower one. Similarly we compared the action on two eggs put side by side between the poles of coil no. 2, one in the centre and the other next to it. If the gradient is decisive it may be expected that a significant difference in the percentages of abnormal divisions appears between eggs put in these two situations. This is indeed the case, as shown in figure 4. A greater range of different gradients was achieved by holding the supply at 26 mA and reducing the pole gap from 2 mm. to 1 mm. and 0.5 mm. The results are represented in figure 5.

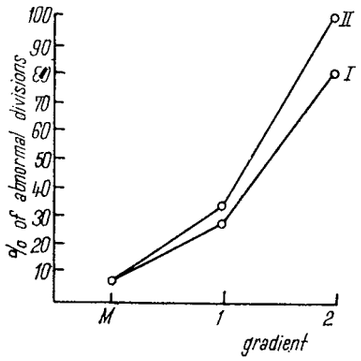


Fig. 4. — Variation of the percentage of abnormal divisions with the gradient. I, in the case of eggs treated one near the other in electromagnet no. 2 ; II, in the case of eggs treated one on top of the other in electromagnet no. 1. *1*, % of abnormal divisions in the lower resp. excentric cells ; *2*, % of abnormal divisions in the upper resp. central eggs. The differences as compared with M (control) are statistically significant ($p<0.01$).

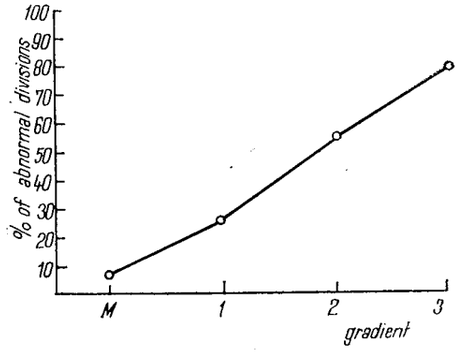


Fig. 5. — Variation of the percentage of abnormal divisions with the gradient. M, control ; *1*, *2*, *3*, gradients corresponding to distances of 0.5, 1, 1.5 mm. between the poles of electromagnet no. 2.

The positions of the different gradients on the abscissa are arbitrarily taken. It can be seen, however, that between field gradient and percentage of abnormal divisions there exists a direct proportionality. Thus it seems that, at least in this case, the field gradient is mainly responsible for the observed effect.

Changes in N—S polarity did not interfere with the result of the treatment. Neither are there modifications due to their orientation with respect to the geomagnetic field in both coils.

If the action of the MF consists in reducing the rotational diffusion of the macromolecules, then it should be inversely proportional to temperature. We did not find, however, any difference between the percentages of abnormal divisions of eggs treated at 28°C and 18°C, although the duration of the first two cell cycles lasted two hours longer at 18°C. Thus it seems that the action of the MF is linked to some specifical periods during which the subsequent cell division is prepared and does not so much depend on the entire time of exposure.

The next problem was to see which period, namely interphase, mitotic stages or cytokinesis may be particularly sensitive to the action of MF. The marked modifications of the egg surface during the first cell cycles described in detail by Huber [8] and Lehmann [9] allows the distinction between interphase and mitotic stages. Based on these characteristics we subdivided the period between the first reductional division and the second mitotic division into equal periods of 80 — 90 minutes of treatment. Period I lasted from the end of the first reductional division until the appearance of the division protuberances during the anaphase of the second reductional division. Period II comprised the anaphase and the telophase of the second reductional division, the formation and fusion of the pronuclei. Period III lasted from the disappearance of the division protuberances up to the onset of furrowing, that means interphase, prophase, metaphase of the first mitotic division. During period IV we treated eggs with well-formed polar plasmas until the formation of the AB cell, the treatment comprising metaphase, anaphase and telophase of the first mitotic division. In period V the first cytokinesis was treated, from the appearance of the furrow until the formation of the AB cell. The VIth period lasted from the rounding up of the two cells during the prophase until the beginning deformation of the CD cell during the anaphase, whereas the seventh period lasted from the deformation of the CD cell until the cytokinesis of the second mitotic division, inclusively.

The result of the treatment with MF during different periods of interphase, mitosis and cytokinesis on the first and second mitotic division is reproduced in figure 6. It can be seen that the greatest effect on the

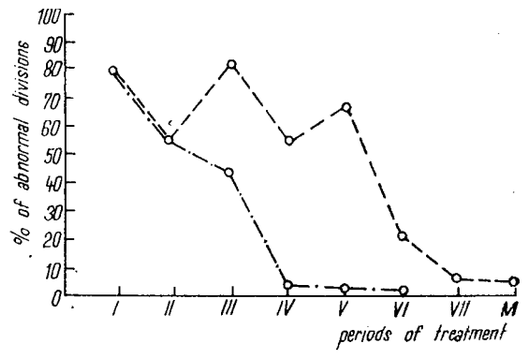


Fig. 6. — The influence of treatment during different periods on the first (—.—.) and second (— — —) division M, control.

first division is obtained during the period between the two reductional divisions. The percentage of abnormal divisions remains significantly higher than in the control also after treatment during period III. Beginning with the IVth period the treatment shows no effect. This means that the disorder of cell division is not produced by altered processes during mitosis and cytokinesis, but that the MF interferes with some processes during interphase or perhaps prophase, responsible for the preparing of a normal division. The fact that the action of the MF is linked to some processes during interphase until prophase is even more clearly shown by the influence of the treatment during different periods on the second mitotic division. Divisions are disturbed only in those cases in which the treatment was performed on eggs before the second reductional division or during periods which correspond mainly to the interphase of the first and second cell cycles. Periods of mitotic activity or cytokinesis are far less sensitive to MF.

For both the first and the second division, it is possible to establish a time before the onset of cleavage after which it is no more possible to induce any modification of the subsequent division through MF. The situation is similar to the description of a "transition point" for various inhibitors of cell division. This point corresponds approximately to the prophase.

The examination of paraffin sections revealed a visible effect of the MF on the structure of the spindle. In treated eggs there is no fibrilar material formed, the spindle is lacking, the chromosomes, however, are well formed.

DISCUSSION

In several cases of treatment with MF it was possible to establish a correlation between the observed effect and field or gradient intensity. For the case described in the present paper no direct correlation between field intensity and perturbation of division could be found. On the contrary, the results strongly suggest a connection between gradient and the observed effect. The fact that the gradient is decisive can probably be best explained through the deviation forces exerted on charged particles. The lack of any differences in the action of the MF on changing the magnetic poles makes the effect of possible differences in the anisotropy of molecular dipoles unlikely. The same seems to be true for the influence of different temperatures on the MF effect.

It is worth mentioning that the expression of the influence of the MF on the first divisions of the Tubifex egg presents great likeness with the picture appearing after treatment with chemical and physical agents. Thus, after treatment with colchicine or theobromine the cleavage also takes place under very strong deformation of the egg cortex [5] [11]. The same is true for mechanical actions (unpublished data and [17]). A "transition point" is also frequently appearing, as well as the interaction of different agents, such as colchicine [4] [20], high pressure [16], temperature [16], etc., with the formation of the MA microtubules. This obviously does not mean that all these agents act on the same processes.

It rather seems that the succession of processes leading to the preparation of a normal cell division, for which a condition is the formation and normal functioning of the microtubular apparatus, may be disturbed by very different alterations of the internal conditions. At the same time the agents may have different points of action. Decisive for the similar aspect of the cell division are probably the similar reactions of the cell against various disturbing influences.

In the case of the action of the MF on the first cleavage divisions of the Tubifex egg, the visible effect of the MF seems to consist primarily in an inhibition of the normal formation of the division spindle. One might presume that the gradient to which the egg cells are exposed prevent the polymerization of a microtubular precursor; leading to the orientation of charged particles, it might hinder their free interaction. Once the spindle microtubules formed, the action of the gradient would cease, which would correspond to the transition point.

Eggs treated even before the first or second reductional divisions exhibit abnormal or blocked mitotic divisions, as shown in figure 6. This means that the alterations produced by the MF in a material necessary for the first cleavage and present already in the unfertilized egg are transmitted along several cell cycles. This again may point to the MF interaction with some spindle precursors which are known to be present in the unfertilized egg [7] [15] [20].

ACKNOWLEDGMENTS

I am indebted to T. Naum, from the Centre of Physical Research, for many discussions and advices in designing the electromagnets.

REFERENCES

1. Barnothy J. M., *Biological Effects of Magnetic Field*, Plenum Press, New York, 1964.
2. — *Abstr. Third Intern. Biomagnetic Symposium*, Univ. of Illinois, Chicago, Illinois, 1966, 1.
3. Beischer D. E., Cowart G. S., U. S. Nav. Acrosp. Med. Inst. Nami, 1970, **1105**, 1.
4. Borisy G. G., Taylor E. W., J. Cell Biol., 1967, a, b, **34**, 525.
5. Brandsch R., Jitariu P., Rev. roum. Biol. Zool., 1971, **16**, 215.
6. Dunlop D. W., Schmidt B. L., *Abstr. Third Intern. Biomagnetic Symposium*, Univ. of Illinois, Chicago, Illinois, 1966, 61.
7. Frankel J., J. Cell Physiol., 1969, **74**, 326.
8. Huber W., Rev. suisse Zool., 1946, **53**, 468.
9. Lehmann F. E., Rev. suisse Zool., 1946, **53**, 475.
10. — Exp. Cell Res., 1958, suppl. 6, 1.
11. — Ergebn. Biol., 1964, **27**, 117.
12. Levengood W. C., Nature, 1966, **209**, 872.
13. Perakis N., C. R. Acad. Sci. Paris, 1939, **208**, 1686.

14. Pumper R. W., Barnothy J. M., *Abstr. Third Intern. Biomagnetic Symposium*, Univ. of Illinois, Chicago, Illinois, 1966, 53.
15. Sisken J. E., Wilkes E., J. Cell Biol., 1967, **34**, 97.
16. Tilney L. G., Marsland D. J., J. Cell Biol., 1969, **42**, 170.
17. Tokumitsu T., Maramorosch K., J. Cell Biol., 1967, **34**, 677.
18. Valentinuzzi M., Terraresi R. W., Vasquez T., *Abstr. Third Intern. Biomagnetic Symposium*, Univ. of Illinois, Chicago, Illinois, 1966, 49.
19. Valentinuzzi M., Vasquez T., An. Soc. Cient. Argentina, 1968, **185**, 3.
20. Zimmerman A. M., Zimmerman S., J. Cell Biol., 1967, **34**, 483.

Biological Research Centre
Jassy

Received November 2, 1971

THE TOXIC EFFECT OF *ERWINIA CHRYSANTHEMI* CULTURE FILTRATES UPON SOME GUINEA-PIG LIVER ENZYMES

BY

DRAGOŞ SCRIPCARIU, RADU MEŞTER and ION LAZĂR

The effect of *E. chrysanthemi* culture filtrates upon some respiratory enzymes (alpha-glycerophosphate dehydrogenase, succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase and cytochrome oxidase) was studied by disk electrophoresis and on histochemical preparations. Under the influence of toxin, a strong repression of enzymatic activity is induced, the pattern of zymograms being considerably modified, the activity of some isozymes completely abolished and the activity of the remaining ones reduced. The data obtained in this paper indicate a strong action of toxin upon the hepatic cell metabolism.

The thorough studies on the zoopathogenic action of phytopathogenic bacteria of the genus *Erwinia*, perfomed by Popovici and Lazăr [20] and Săvulescu and Lazăr [21], as well as those concerning the nature of toxins from culture filtrates of different species of *Erwinia*, lead to the conclusion that a series of germs species of this group have the capacity of producing strong septicaemiae and intoxication, which end with the death of test animals.

The strongly noxious action of these bacteria, as well as the nucleoproteinic action of the toxins elaborated by them [10—12] determined us to begin a detailed study on the latter's mode of action upon the metabolism of hepatic and renal cells.

In previous works, we studied, under the histochemical aspect, the effect of toxins upon certain chemical and enzymatic constituents of the guinea-pig liver and kidney: the electrophoretic patterns of carbonic anhydrase, phosphate hydrolase, acid phosphatase, thiamine pyrophos-

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, N° 2, p. 131–136, BUCAREST, 1972

phatase, ATP-ase and certain glycolytic enzymes [22—25]. The data obtained show that the toxin has a strong toxic action upon the studied organs inducing grave morphological and metabolical modifications.

In the present work we expose the data concerning the action of *E. chrysanthemi* toxic filtrates upon respiratory enzymes.

MATERIAL AND METHODS

Experiments were performed on guinea-pigs of an average weight of 150 g. The animals were injected intraperitoneally with 1.5 ml. doses, at 4 hours' interval. Animals were sacrificed 12 hours after the last dose.

The toxin was obtained according to the technique described by us in a previous work [22]. The liver homogenate was prepared in conditions described previously [22].

Electrophoresis was carried out for 3 hours at 3.12 mA/tube, on polyacrylamide gel, according to disk electrophoresis system [3], using aliquots of 0.1 ml. supernatant.

After electrophoresis, the gels have been treated for 30 min. with tris-HCl 0.2 M buffer, pH 7.6. For the detection of enzymatic activity, the gels were submitted to incubation in media prepared in tris-HCl 0.2 M, pH 7.6; for the rest of components, the specific techniques of detection of each enzyme was respected. Histochemical preparations were made on unfixed liver sections, performed at freezing microtome [2]; the incubation media have been the same as in polyacrylamide gels.

Alpha-glycerophosphate dehydrogenase was incubated in medium prepared by Lush [17], in which the lactate was replaced by alpha-glycerophosphate with the same molarity; succinate dehydrogenase has been detected in medium prepared according to Nachlas et al. [18] [19], malate dehydrogenase in the medium recommended by Davidson and Cartner [4], isocitrate dehydrogenase has been evidenced in Farber's medium, in which NADP was replaced with NAD [9]. In order to make evident the cytochrome oxidase the method of Nachlas et al. was used [18].

The isoenzymatic bands were numbered from anode towards cathode, according to Webb's method [30].

RESULTS

Alpha-glycerophosphate dehydrogenase. The zymogram of this enzyme in liver homogenate of control animals exhibit 4 isoenzymatic bands: bands 1, 3 and 4 have a medium reaction while the 2nd band is narrower and has a more intense reaction. In the liver homogenate of treated animals, only the enzymatic fractions 3 and 4 appear, whose enzymatic activity is unmodified. The enzymatic activity of the first two fractions, with the greatest anodic rate, disappears under the influence of bacterial toxin (Fig. 1).

Following the action of toxin on the histochemical preparation, the alpha-glycerophosphate dehydrogenase still remains active in the zone of the Kiernan spaces, as well as in some hepatocytes in the proximity of the central lobular vein. In the lobe mass, the reaction appears completely inhibited (Fig. 6).

Succinate dehydrogenase. The electrophoretic analysis of the enzyme from liver homogenate of normal and treated animals presents a pattern

similar to the enzyme described above. The normal liver homogenate presents 4 distinct isoenzymatic fractions with a great enzymatic activity. Under the influence of toxin, the enzymatic spectre undergoes great modifications; besides the fact that the first two bands disappear from the spectre, as likewise in the case of the first enzyme, bands 3 and 4 undergo a reduction of enzymatic activity, this being more evidently recorded in the case of fraction 4 (Fig. 2). Histochemical reaction on treated animal liver is only preserved in hepatocytes around the Kiernan spaces, the rest of the lobule presenting a negative reaction (Fig. 7).

Malate dehydrogenase. The malate dehydrogenase of vertebrates is described in the literature as presenting two forms, with different intracellular localization and different electrophoretic mobilities [5—8] [26] [27] [29]. The cytoplasmic form has a greater electrophoretic mobility than the mitochondrial one.

In the zymograms obtained by us from liver homogenate of control animals, one band corresponding to the cytoplasmic form and two bands with slow migration, corresponding to the mitochondrial one, were isolated. The bands corresponding to the mitochondrial form have a much greater reaction intensity than the fraction corresponding to the cytoplasmic form. These results are similar to those obtained by Thornber et al. [28] in analysing the malate dehydrogenase from guinea-pig liver by starch gel electrophoresis. Under the toxic action of *E. chrysanthemi* culture filtrate, hepatic malate dehydrogenase undergoes important modifications. The activity corresponding to the cytoplasmic form is completely inhibited, while from the mitochondrial isozymes only the first band is preserved (Fig. 3).

Malate dehydrogenase in treated liver slices presents a slight diffuse reaction without a specific localization in the soluble structure. The hepatic tissue presents a necrotic aspect (Fig. 8).

Isocitrate dehydrogenase. From the normal liver homogenates, 3 isozymes with slow migration rate were isolated, being situated in the cathodic half of the gel. The enzymatic activity of the bands decreases from the fraction with the slowest anodic migration rate towards the most mobile one. In treated animals, 4 isozymes were isolated from the liver homogenate, the supplementary one presenting the highest enzymatic mobility and activity. The other 3 fractions present a mobility similar to the normal but manifest a lower specific activity (Fig. 4).

On histochemical preparations, a reaction appears with a mosaic aspect, cells with an intense positive reaction mixed with cells devoid of enzymatic activity unhomogeneously distributed throughout the entire lobule mass (Fig. 9).

Cytochrome oxidase. From the normal liver homogenates 3 major fractions are isolated, of which fractions 1 and 2 present one subfraction each. The first two fractions present a more intense enzymatic activity. The enzymatic activity of subfractions 1 and 2 disappears from the homogenates of treated animals, the rest of the zymogram remaining unaltered (Fig. 5).

On histochemical preparations the cytochrome oxidase reaction remains intense. A lower enzymatic activity is recorded in the peripheral part of the lobular parenchyma (Fig. 10).

DISCUSSION

The data obtained by us evidence the toxic effect exerted by *E. chrysanthemi* culture filtrates upon the enzymatic chain of cellular oxidative phosphorylation. Enzymatic inhibition induced by bacterial toxin has as a consequence, a reduction of the cell respiratory activity and a decrease of the cellular energy metabolism. This fact is rendered evident on histochemical preparations in a previous work [22]. The reduction of the activity of respiratory enzymes chain in hepatocytes is accompanied by a mitochondrial fragmentation.

Similar results were obtained by Buris et al. [1] who, working with a staphylococcic enterotoxin, observed important modifications of the glycolytic metabolism of the cat striated muscle, liver and intestine. The authors noticed a great perturbation of the activity of glucose-6-phosphatase, acid and alkaline phosphatase, phosphorylase and aldolase, which modify the glycolytic metabolism, inducing the blocking of hepatic and muscular glycogenolysis and leading to the loading of these tissues with glycogen.

Important variations of some enzymes from different organs were obtained also by Jonek et al. [13] in the case of some lead intoxications, and by Jonek et al. [14] [15] and Kaminski et al. [16] in the case of benzene intoxications. The histoenzymatic observations express a lesion of cellular membranes with an alteration of enzymes connected with them, as well as an activation of lysosomal hydrolases, a process which finally lead to the necrosis of the affected tissues.

Scripcariu et al. [23] [24] [25] described the modifications induced by the toxic filtrates of bacterium cultures of the *Erwinia* group, upon the enzymes connected with the active transport of membrane and ionic equilibrium of the cell. The present report completes the study of the inhibiting action of this toxin with the effects upon the alpha-glycerophosphate dehydrogenase enzyme, as well as of enzymes of the Krebs cycle, demonstrating the lowering of mitochondrial metabolism.

Cytochrome oxidase presents a smaller sensitivity to the action of the toxin, the reaction intensity of the main isoenzymatic bands being little affected. Histochemical preparations confirm the results obtained by electrophoresis. In the hepatic lobules large zones were observed where enzymatic reaction was reduced; in certain cases it was maintained in the zone of Kiernan spaces. Cytochrome oxidase is less affected by bacterial toxin, presenting only an inhibition zone at the periphery of the lobule.

The mechanism of the action of *Erwinia* toxin culture filtrates upon the cellular functions is still difficult to explain. However, we are attracted by the hypothesis that toxin would act directly on the membranes, modifying their permeability. It was pointed out that toxin penetrates into hepatic cells and epithelia of renal tubule, where it could be revealed by fluorescence. A second possibility i sthat toxin interacts with the enzymes or with the genetic mechanism of protein synthesis. The disappearance of some enzymatic fractions from the zymogram, or the appearance of some others with electrophoretic properties completely different from the normal, are of considerable interest in this connection.

CONCLUSIONS

The toxic effect of *Erwinia chrysanthemi* culture filtrates upon the respiratory metabolism of hepatic cells is manifested by an inhibition of enzymes connected with this process and accompanied by the disappearance of certain isoenzymatic fractions electrophoretically revealable.

In the electrophoretic pattern of alpha-glycerophosphate dehydrogenase and succinate dehydrogenase, under the influence of toxin two fractions with great electrophoretic migration disappear. Malate dehydrogenase from liver homogenate of treated animals presents a single band with weaker activity as compared with the normal one.

Isocitrate dehydrogenase from the liver of treated animals presents a supplementary electrophoretic band, while cytochrome oxidase undergoes small modifications.

The histochemical preparations for the enzymes detection confirm the electrophoretic results, evidencing wide inhibition zones of enzymatic activity in the hepatic lobule. Cytochrome oxidase seems little affected by toxin.

The results so far obtained on crude enzyme preparations indicate that toxins act upon the structure of cellular membranes or upon the protein biosynthesis, leading to the appearance or disappearance of some isozymes.

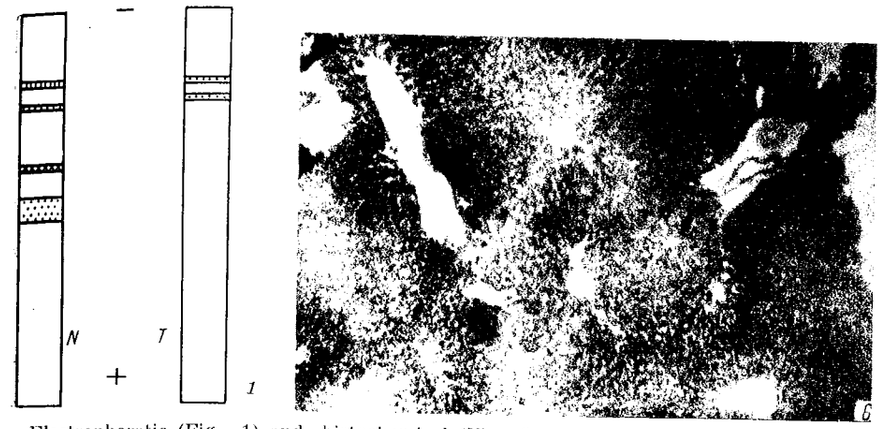
REFERENCES

1. Buris L., Szrlgy I., Zsigmound K., 1969, **34**, 183.
2. Burstone M. S., *Enzyme Histochemistry and its Application in the study of Neoplasm*, Acad. Press, 1962, 514 p.
3. Davis J. B., Ann. N.Y. Acad. Sci., 1964, **121**, 1964.
4. Davidson G. R., Cartner A. J., Science, New York, 1967, **157**, 1569.
5. Delbruck A., Zebe E., Bucher T., Biochem. Z., 1959, **331**, 273.
6. Delbruck A., Schinnasek H., Bartsch T., Biochem. Z., 1959, **331**, 297.
7. Dohert Y. D., J. biol. Chem., 1961, **236**, 1980.
8. Englard S., Brieger H. H., Biochim. biophys. Acta, 1962, **56**, 571.
9. Farber E., Bueding E., J. Histochem. Cytochem., 1956, *4*, 357.
10. Grou E., Lazăr I., Microbiologie, S.S.B., 1969, *1*, 217.
11. Grou E., Lazăr I., St. Cercet. Biol., Ser. Bot., 1969, **21**, 307.
12. Grou E., Microbiologie, S.S.B., 1970, *2*, 17.
13. Jonek J., Kosmoder S., Jonek T., Kaiser J., Parodontologic and Academic Rev., 1968, *2*, 19.
14. Jonek J., Kaminski M., Karbowski A., Grazibek H., Medicyna Pracy, 1969, **20**, 504.
15. Jonek J., Kaminski M., Karbowski A., Medicyna Pracy, 1969, **20**. 7.
16. Kaminski M., Karbowski A., Jonek J., Folia Histochem. Cytochem., 1970, *8*, 63.
17. Lush E. I., Comp. Biochem. Physiol., 1970. **32**, 23.
18. Nachlas M. M., Tson K. C., de Souza E., Cheng C. S., Seligman A. M., J. Histochem. Cytochem., 1957, *5*, 420.
19. Nachlas M. M., Crawford D. T., Goldstein D., Seligman A. M., J. Histochem. Cytochem., 1958, *6*, 445.
20. Popovici I., Lazăr I., Rev. roum. Biol. Sér. Zool., 1964, **9**, 147.

21. SĂVULESCU A., LAZĂR I., St. Cercet. Biol., Ser. Bot., 1966, **18**, 183.
22. SCRIPCARIU D., LAZĂR I., Microbiologie, S.S.B., 1969, *1*, 189.
23. SCRIPCARIU D., LAZĂR I., Microbiologie, S.S.B., 1970, *2*, 21.
24. SCRIPCARIU D., LAZĂR I., POPA A., CISMAȘ A. I., Microbiologie, S.S.B., 1970, *2*, 37.
25. SCRIPCARIU D., LAZĂR I., CISMAȘ A. I., POPA A., Microbiologie, S.S.B., 1970, *2*, 29.
26. SIEGEL L., ENGLARD S., Biochim. biophys. Acta, 1961, **54**, 67.
27. SRAGA E., Arch. Biochem. Physiol. Biophys., 1965, **109**, 57.
28. THORNBER E. J., OLIVIER I. T., SCUTT P. B., Comp. Biochem. Physiol., 1968, **25**, 973.
29. THORNE C. J., GROSSMAN L., KAPLAN O. N., Biochim. biophys. Acta, 1963, **73**, 193.
30. WEBB E. C., Experientia, 1964, **20**, 592.

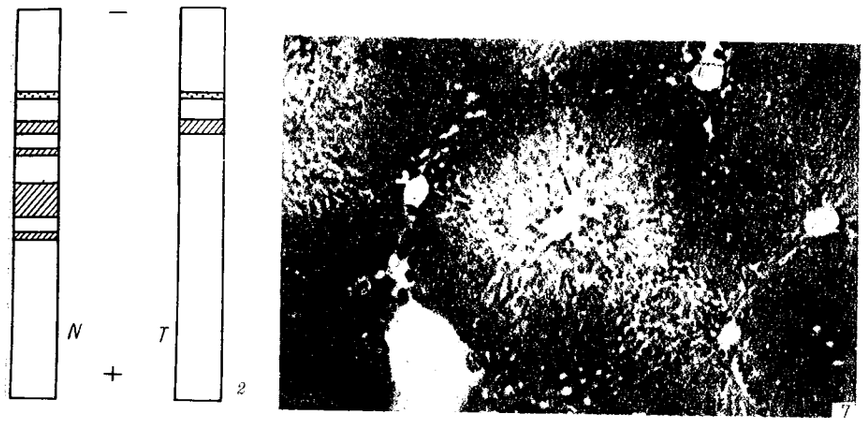
Facully of Biology
and
The "Traian Săvulescu" Institute of Biology

Received June 12, 1971



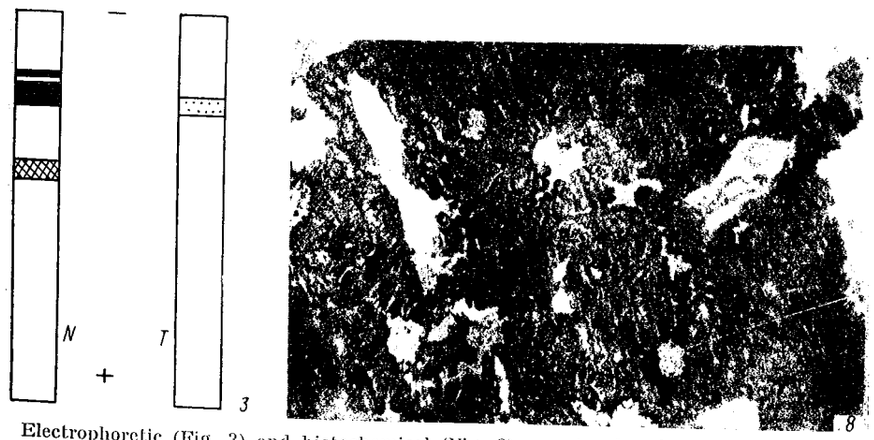
Electrophoretic (Fig. 1) and histochemical (Fig. 6) aspects of alpha-glycerophosphate dehydrogenase, tris-HCl 0.2 M, pH = 7.6, buffer in medium with DPN (diphosphopyridine nucleotide).

N = Zymogram of liver homogenate obtained from normal animals.
T = Zymogram of liver homogenate obtained from treated animals.



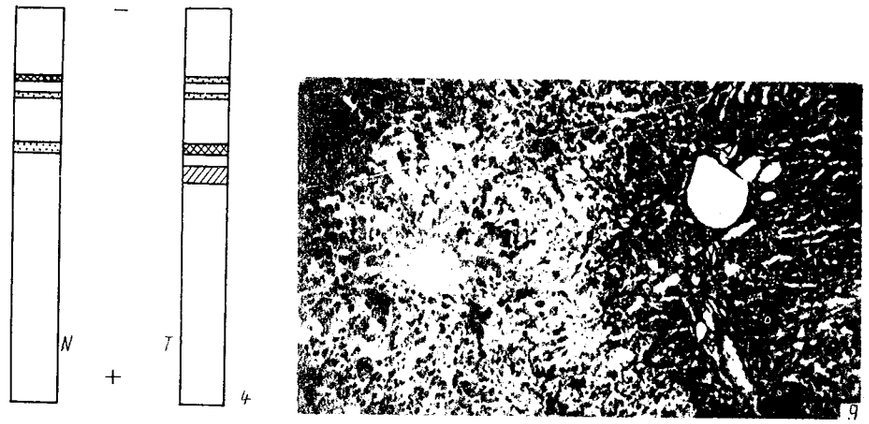
Electrophoretic (Fig. 2) and histochemical (Fig. 7) aspects of succinate dehydrogenase, tris-HCl 0.2 M, pH = 7.6, buffer in medium with DPN.

Same legend as for figure 1.



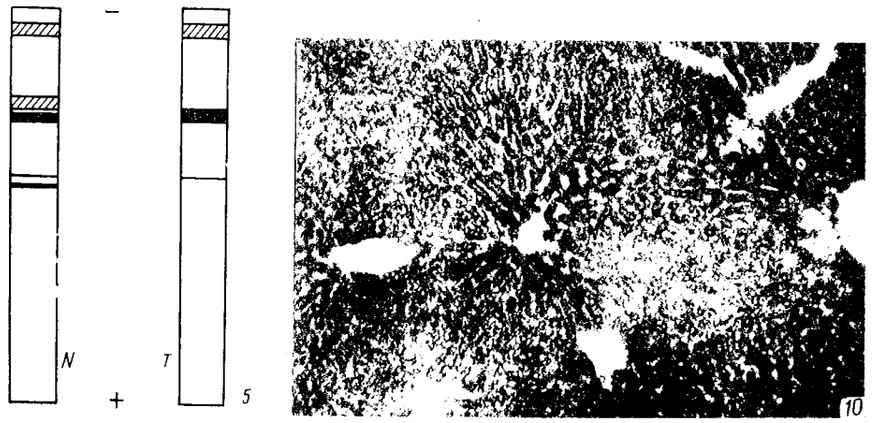
Electrophoretic (Fig. 3) and histochemical (Fig. 8) aspects of malate dehydrogenase, tris-HCl 0.2 M, pH = 7.6, buffer in medium with DPN.

Same legend as for figure 1.



Electrophoretic (Fig. 4) and histochemical (Fig. 9) aspects of isocitrate dehydrogenase, tris-HCl 0.2 M, pH = 7.6, buffer in medium with DPN.

Same legend as for figure 1.



Electrophoretic (Fig. 5) and histochemical (Fig. 10) aspects of cytochrome oxidase, reaction "NAD-M", with alpha-naphthol and p-amino-dimethyl aniline 0.1 M, pH = 7.4, phosphate buffer.

Same legend as for figure 1.

THE BIOSTRUCTURE AS A CELL ULTRASTRUCTURE*

BY

E. MACOVSCHI

Biostructure existence within living tissues was proved by means of high pressure squeezing and nuclear magnetic resonance methods. In the present paper two indirect procedures able to evidence biostructure by means of electron microscopy are proposed.

The present biology is dominated by the molecular conception according to which both living and dead cells include the same structures; each of them has the same specific chemical composition and the same characteristic make-up of the matter which is in the same state. Consequently, according to the molecular conception, both living and dead cells have the same make-up, and cell death is not accompanied by modifications at cell structure level. We are so persuaded about the justness of this statement that we forget it is only a postulate the validity of which was not proved so far. However, starting from the idea that death is not accompanied by a modification of cell structure, certain new points of view were reached, which affect the explanation of experimental results. For instance we believe that optic or electron microscope pictures obtained on biological materials killed, fixed, cut and treated with various reagents and dyes reflect structural realities in living biological materials, because we admit that dead cells maintain the living cell structures. We also state that intracellular formations obtained after homogenization and fractional centrifugation of killed biological materials also occur within living cells, because we believe that they have the same form and structure as outside the cells.

* Communication presented on November 22, 1971 at the Colloquium "Ultrastructure studies in biology" (Bucharest) sponsored by the Department of biological sciences of the Academy of the Socialist Republic of Romania.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 17, Nº 2, p. 137–139, BUCAREST, 1972

In the light of the recently advanced biostructural conception [3] [4] the situation appears quite differently. According to this conception, living cells deeply differ from dead ones because a part of the matter they are made up of has a richer energetic state than the common one and is integrated into a quite particular continuous structure, peculiar to life and occurring only within living beings. At death, this structure, called "biostructure", breaks down and sets free the components it is made up of. Thus, in contrast to the molecular conception stating that cell death is not accompanied by structural modifications, the biostructural conception states that just during death essential structural modifications occur within cells; owing to the latter, the make-up of dead cells deeply differs from that of living cells. For this reason, of course, the explanation of experimentals results leads to other conclusions and opens quite new prospects to the understanding of biological phenomena.

But, along with biostructured matter, living cells also contain common, molecular matter, partly represented by numerous organic and mineral compounds dissolved within cell free water. Within this "intracellular solution" the energy-yielding cell biochemistry, so necessary to the formation of biostructured matter and to the maintenance of biostructure integrity, is developing; in turn, biostructure, along with other factors, participates in cell biochemistry regulation. In the framework of this interdependence, some spatial relationships between biostructure and the intraplasmatic solution can be visualized by admitting that biostructure occurs as a spongy mass crossed by anasthomosed spaces filled with intracellular solution. Thus, biostructure forms a real ultrastructure of living cells which, however, deeply differs from any other cell structures and ultrastructures by its nature as well as by its capacity of breaking down at cell death. For this reason, the existence of biostructure was not yet detected either by optical or electron microscopy.

In turn, recent experimental studies performed by means of other investigation methods, such as squeezing of biological material at high hydrostatic pressures [8] [9] as well as high resolution and spin echo nuclear magnetic spectroscopy (NMR) [11] have proved the existence of biostructured matter within living tissues [6]. Other investigations have confirmed different premises of the biostructural conception concerning, for instance, the release of biostructured matter compounds under a slow death [8] or in the presence of metabolic inhibitors [2] [13] [14], biostructure breakdown at high temperatures [10], the partial and reversible breakdown of biostructured matter under electrical [1] or ultraviolet ray stimulation [7] of living tissues, etc. It was found that nucleic acids [12] are among biostructured matter compounds, a fact highly interesting for the problem of protein biosynthesis regulation by the intervention of biostructure. Moreover, a hypothesis was advanced, according to which intracellular formations are generated by the biostructured matter while some of them; viz. ribosomes, polysomes and others, represent fragments of biostructure [5]; the verification of this hypothesis is now in progress.

But, as biostructured matter exists, maybe electron microscopy could, nevertheless, permit its detection by indirect procedures.

For the time being, two such procedures could be visualized; however, we are not quite sure about their issue, owing to the complexity of the problem.

One of them could be based on the well-known fact that living organisms frozen under certain conditions still live after thawing. It means that under such conditions their biostructure keeps untouched. It is very likely that, by perfusing with suited solutions chilled tissues, a technique able to detect biostructured matter could be advanced.

Another procedure could be based on the observation that during biostructured matter breakdown, the process of splitting occurs in different ways, depending on the conditions under which cells are killed. Thus, the comparative study of electron microscope pictures of one and the same biological material killed in different ways, namely non-treated or treated before killing with substances capable of influencing the state and the integrity of biostructure, could yield some data liable to confirm the existence of biostructured matter.

REFERENCES

1. Cornoiu I., St. Cercet. Biochim., 1971, **14**, *1*, 17.
2. Elefterescu V., St. Cercet. Biochim., 1970 **13**, *3*, 261.
3. Macovschi E., Rev. Chim., 1959, *4*, *1*, 11.
4. — *Biostructura*, Ed. Acad. Bucharest, 1969.
5. — Rev. roum. Biochim., 1969, **6**, *1*, 3.
6. — St. Cercet. Biochim., 1971, **14**, *3*, 239.
7. Macovschi E., Ceauşescu S., St. Cercet. Biochim., 1968, **11**, *4*, 331.
8. Macovschi E., Marineanu I., Rev. roum. Biochim., 1967, **4**, *4*, 235.
9. Macovschi O., Rev. roum. Biochim., 1970, **7**, *3*, 201.
10. Mihăescu A., St. Cercet. Biochim., 1970, **13**, *3*, 281.
11. Mosora F., Rev. roum. Biochim., 1972, **9**, *1*, 51.
12. Muşolan C., St. Cercet. Biochim., 1972, **15** (in press).
13. Rabega C., Stănescu R., Ann. Univ. Bucureşti, 1972 (in press).
14. Rabega M., St. Cercet. Biochim., 1971, **14**, *1*, 79.

University of Bucharest
Department of Biochemistry

Received December 17, 1971

AVIS AUX AUTEURS

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

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

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

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, les tableaux et les graphiques sera évitée. Les références bibliographiques citées par ordre alphabétique des auteurs comporteront le nom de l'auteur, l'initiale, le titre de la revue, abrégé conformément aux usances internationales, l'année, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé, de maximum 10 lignes. Les textes des travaux ne doivent pas dépasser 15 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures).

Les auteurs ont droit à 50 tirés à part gratuits.

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

La correspondance relative aux manuscrits, à l'échange de publications, etc. sera adressée au Comité de rédaction, 296, Splaiul Independenţei, Bucarest.

PRINTED IN ROMANIA