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# REVUE ROUMAINE DE BIOLOGIE



## SÉRIE DE BIOLOGIE ANIMALE

TOME 25, N° 2 juillet-décembre 1980

## SOMMAIRE

# CONTRIBUTION À LA CONNAISSANCE DES MONOKONOPHORA *(CRUSTACEA, TANAIDACEA)* DE LA MER MÉDITERRANÉE; DESCRIPTION DE DEUX ESPÈCES NOUVELLES, *APSEUDES SICILIANUS* sp.n. ET *A. MISARAI* sp.n.

PAR

M. BĂCESCU

On the basis of a material collected in 1977 by the R/V Calypso during its campaign in the Mediterranean and of a small collection of spongicolous Tanaidacea, two new species — *Apseudes sicilianus* and *A. misarai* are described. *A. ostroumovi* is new for the west of the Mediterranean and the Adriatic; *A. intermedius* is new for the west of the Mediterranean (Genova, Napoli, Bari). For *Tuberapseudes echinatus*, new morphological details are given that make the sub-genus *Tuberapseudes* Băc. & Guțu be in good reason considered a genus.

Ayant l'occasion d'étudier une petite collection de Tanaidacés envoyée par le Dr. H. Zibrowius de la Station Maritime d'Endoume (France) — Tanaidacés récoltés par lui-même dans le Golfe de Gènes ou par la « Calypso », dans d'autres parties de la Méditerranée — j'ai examiné aussi quelques autres échantillons se trouvant dans les collections du Muséum « Grigore Antipa », que voici :

— Tanaidacés commensaux aux éponges *(Agelas* surtout) de Bari (SW de la mer Adriatique), envoyés par le Professeur Michel Sara et capturé en 1968.

— Quelques échantillous capturés par moi-même à Monaco en 1968, à Florida en 1968 ou à Dakar (1972).

A l'examen de ces échantillons, on peut faire les observations suivantes :

### 1. ***Tuberapseudes echiatus*** (Băc. & Guțu 1971)

(Fig. 1 A–C)

Bien que tant les spécimens d'Espagne que ceux de l'Adriatique capturés par la Calypso correspondent au type (Sars 1882), nous pouvons tout de même signaler quelques nouveaux détails morphologiques qui justifient de considérer cette espèce non seulement comme le représentant d'un sous-genre, tel que nous l'avons considéré autre fois (v. Băcescu & Guțu, 1971, p. 66), mais comme le représentant d'un bon genre.

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Premièrement, la présence, sur le péréiopode III aussi, d'une épine coxale typique (fig. 1 A) (♂ ♀) plus pointue que la plaque coxale du péréiopode II (fig. 1 B) ; deuxièmement, la présence d'autres épines coxales, plus petites, fixées cette fois-ci, sur la partie postérieure des longues coxas des péréiopodes V — VII.

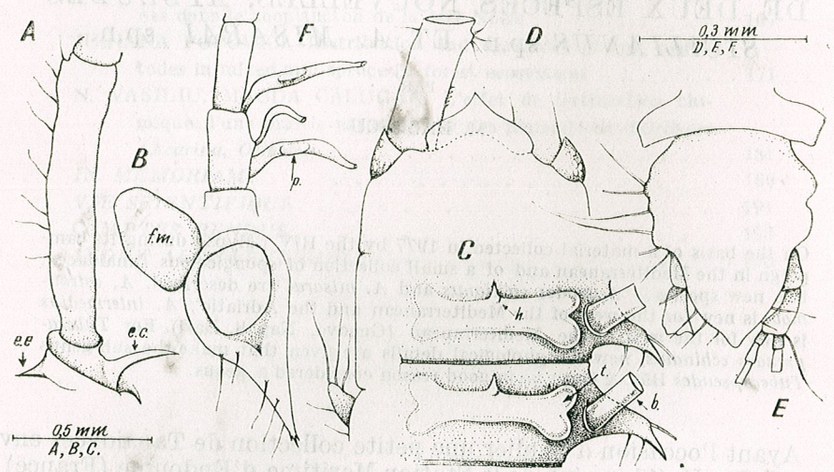


Fig. 1. — A — C, *Tuberapseudes echinatus*. A, plaque coxale du troisième péréiopode ; e.c., épine coxale ; e.e., épine épimérale antérieure ; B, id., du II^e^ péréiopode, f.m. ébauche marsupiale ; C, les sternites bituberculés des pléonites II et III ; b, la base d'un pléopode ; h, épine hyposphaeniale minuscule ; t, tubercule double des plaques stérales.
D — F, *Apseudes intermedius*. D, partie antérieure ; E, extrémité caudale ; F, deux articles de l'antenne, avec des formations (p) insolites (parasites ?).

En commençant avec le péréionite V, les coxas deviennent des bracelets larges qui dépassent de beaucoup les épimères et laissent voir clairement leur épines postérieures, elles aussi n'étant figurées ni chez Sars (1882) ni chez Norman et Stebbing (1886).

Une autre distinction réside dans le fait que certains spécimens ont les tubercules des plaques sternales abdominales bituberculés (h, fig 1C) et sur la plaque sternale, entre ces tubercules, chez 2 spécimens, on remarque des restes minuscules d'épines hyposphéniales (caractère juvénile ou ancestral ?). Les péréionites moyens sont pourvus de 3 épines latérales à cause du fait que sur la partie antérieure de l'épimère apparaît une épine, alors que sur les deux derniers segments, l'épine antérieure se déplace vers le milieu des segments.

Les coxas des péréiopodes III et IV sont des anneaux étroits qu'on ne voit pas d'en haut, tout en étant couverts des épimères et n'ont pas d'épines.

Espèce atlanto-vest méditerranéenne, nouvelle pour les mers Thyrrenienne et Adriatique.



2. ***Apseudes intermedius*** Hansen 1921

(Fig. 1 D — F)

Le fait que dans les captures mentionnées nous avons trouvé cette espèce dans la biocœnose caractéristique de certaines éponges, nous incite à croire qu'elle y mène une vie spongophyle, sinon spongobionte ; tous les individus sont conformes au type (fig. 1 D et E).

C'est un fait confirmé aussi par sa récolte soit en état libre, soit dans les spongiaires non identifiés de quelques-unes de nos anciennes stations de Floride (1968) de Dakar, 20.II.1972, ou de Monaco (26 sept. 1968). Certains ont un drôle d'esthétasques (fig. 1 F). Parasités ?

La vie spongicole de cet *Apseudes* nous conduit à l'idée que sa petitesse même, ses grands chélipèdes ♂ ♀ et, en dernière analyse, son hermaphroditisme, doivent résulter de son processus d'adaptation à ce milieu.

3. ***Apseudes sicilianus*** n.sp.

(Fig. 2 A — K)

*Description* (♀). Longueur 11,5 mm. Corps élancé, fortement aplati dorso-vetralement, rappelant, de profil, la silhouette de l'*Apseudes robustus* Sars.

Carapace large, avec un puissant rostre triangulaire, à peine cordiforme à sa base, légèrement courbé apicalement (fig. 2 A et B). Le premier péréionite libre présente un prolongement postérolatéral en forme de piquant et il est plus de deux fois plus large que longue ; les autres thoracomères s'allongent de plus en plus ; le IV^e^, le V^e^ et le VI^e^ sont nettement plus longs que larges.

Les angles antérolatéraux des autres péréionites s'aplatissent latéralement et finissent aussi en angle aigu (fig. 2 A). La partie postérolatérale de tous les péréionites a un bout courbé, pointu, dirigé caudalement. Le trajet plus foncé du tube digestif et l'épaisseur de la musculature des péréiopodes font un dessin rhomboïdal, caractéristique, sur le fond légèrement transparent des parties latéro-antérieures, aplaties jusqu'à être transparentes.

Les pléonites décroissent en longueur et sont pourvus de prolongements épiméraux poilus, qui finissent par des épines courtes, dirigeés en bas. Le pléotelson est tout aussi long que les 4 derniers pléonites et apparaît comme un tonnelet hirsute, sans aucune épine (fig. 2 $A_1$). Des hyposphénia petits sous tous les pléonites et quelques-uns, d'à peu près la même forme et taille, sur la face ventrale des péréionites (fig. 2 C.)

Le tégument mou, peu poilu, à l'exception du pléon qui est puissamment garni de soies simples, longues, sur les épimères.

Antenne I avec le premier segment basal long, poilu, sans épines ; le reste, déchiré. Antenne II (fig. 2 D) avec une écaille entourée de 14 poils (soies simples) longs et un endopodite de 13 segments. La mandibule avec palpe 3-articulé, fin ; pars incisiva pourvue de 2 dents puissantes, brunes au bout ; une lacinia mobilis forte et une pars mastica-


Fig. 2. — *Apseudes sicilianus* n.sp. A, ♀ vue d'en haut ; B, céphalothorax de profil ; C, les segments thoraciques 3 et 4, de profil afin de permettre l'observation des petites hyposphéniens ; D, antennule ; E, mandibule ; F, extrémité de l'exognathe du maxillipède III ; G, chélipède ; H, péréiopode II ; K, péréiopode VI.



toria large, aplatie à son bout comme un ciseau (fig. 2 F). Entre les palpes de la mandibule, à la partie inférieure de la paroi verticale du labrum (fig. 2 B) fait saille une puissante épine, l'épistome.

La maxillule avec un bouquet d'épines longues sur l'endite externe et un palpe biarticulé armé de 3 soies inégales.

Maxillipède : forme usuelle, avec 5 rétinacules. Epignathe avec une apophyse longue, pourvue de beaucoup de petites épines (fig. 2 F).

Chélipèdes extrêmement fins, avec exopodites et 3 épines puissantes sur l'ischium (fig. 2 G), qui devient très étroit vers la coxa ; les doigts de la pince extrêmement fins et sans aucun tubercule. Le dactyle, en échange, a deux soies simples longues, caractéristiques, subterminales.

Le péréiopode II (fig. 2 H) a un exopodite petit, 3 épines puissantes sur la partie antéro-externe de l'ischium et 9 épines fines sur la marge du propus, très aplatie ; on voit le reste des phanères sur la fig. 2. Le péréiopode VI (fig. 2 K) avec un dactyle long et fin, armé d'une griffe également très fine. Les pléopodes manquent, mais on en voit clairement les traces des 5 paires ; la base de l'uropode (fig. 2 $A_1$) très courte, poilue, le reste déchiré.

*Localité, matériel.* Une seule ♀ avec des ébauches marsupiales, capturée à l'aide de la drague Charcot, à une profondeur de 110 m, à Gela, au sud de Sicile, le 18.11.77 (St. 8), par la Calypso, pendant la campagne de 1977, initiée par J.Y. Cousteau pour constater le processus de pollution de la Méditerranée et des mers annexes.

Holotype ♀ (avec une partie de l'abdomen et des membres détachés) enregistré sous le n° 513, col. Crust. du Muséum « Grigore Antipa ».

*Observations* : On peut comparer l'*Apseudes sicilianus* seulement à *Apseudes robustus* en ce qui concerne l'aplatissement dorso-ventral des péréionites et l'extrême fragilité des appendices (v. Băcescu 1961). Bien qu'il soit seul et incomplet, la morphologie à part de ce Tanaidacé le distingue sûrement de toutes les espèces connues, par la forme et la phanérotaxie des segments du corps.

Les yeux présentent seulement un cercle de petits amas de granules au lieu des omatidies (fig. 1 A).

*Derivatio nominis* : l'espèce a été capturée à Gela, dans les eaux S de la Sicile.

### 4. ***Apseudes ostroumovi*** (Băcescu et Aur. Căraușu 1947)

L'espèce à été constatée dans les eaux de Barcelone (Espagne) et de Split (Jugoslavie) en Adriatique (campagne de la Calypso en 1977) ; c'est pour la première fois qu'on cite en Méditerranée *A. ostroumovi*, espèce qui paraissait endémique pour la mer Noire.

Si nous sommes d'accord avec Lang (1955) que le taxon *Apseudopsis* ne peut pas être valide, même en tant que sous-genre, nous ne pouvons pas souscrire à la synonymie d'*A. ostroumovi* avec *A. acutifrons* proposée par lui. Le fait que la plaque rostrale d'*A. ostroumovi* possède deux plissements latéro-dorsaux — deux goutières ; que son rostre est de beaucoup plus court que la largeur de la dite plaque ; que les épines antéro-latérales

sur les thoracomères font défaut ; que le pléotelson a une forme ± quadrangulaire, pour nous arrêter à ces traits, sont autant de bonnes raisons pour caractériser une bonne espèce.

### 5. *Apseudes misarai* n.sp.

(Fig. 3 et 4)

*Description de la femelle.* Corps six fois plus long que large (fig. 3A). Tégument mou, avec de faibles plis sur la carapace et les thoracomères marqués de deux plis longitudinaux de chaque côté. Carapace à large rostre triangulaire dont le bout obtus est légèrement courbé et finement dentélé (fig. 3 E).

Le thoracomère soudé, très court, ayant les côtes parfaitement lisses (fig. 3 A) ; les thoracomères III — VI présentent chacun une expansion antéro-latérale non épineuse, mais bordée de 3— 4 soies pennées et une autre plus petite postéro-latérale (fig. 3 B) ; sur le dos il n'y a que de rares soies poilues.

Les pléomères ont des épimères longs et clairement tronqués, finissant par 5—6 énormes soies pennées à barbes extrêmement longues. Le pléotelson, plutôt cylindrique, a quelques petits gonflements latéraux — points d'insertion des longues soies et deux autres plus saillants qui servent à l'insertion des courtes bases des uropodes (fig. 3 C) ; chez cet *Apseudes* tout — les derniers péréiopodes, les pléonites, le pléotelson et la base des uropodes — est pourvu d'énormes soies, plus longues que le diamètre du pléotelson. Je souligne qu'il s'agit de soies d'un type peu commun, à de très longues pennae (fig. 3 C, J et la flèche fig. 3 B) de vrais filaments qui forment un tissu inextricable de fils autour du crustacé.

Un épistome (e, fig. 3 E) long et aigu s'élève sur l'anneau au-dessus du labrum, dont la lèvre est linguiforme et hirsute ; les épines hyposphaeniales manquent ; à l'exception de celle située entre les chélipèdes ; il n'y a point d'épines hyposphaeniales même sur les sternites du pléon et entre les derniers péréiopodes (hermaphrodite ?) ; des yeux triangulaires coniques sans épines, mais avec une douzaine d'omatidies, évidentes d'en bas.

Antennules avec les bords internes de la base finement découpés en dents de scie (fig. 3 E et la flèche) ont 12 articles au flagellum court et 18 à celui long. Antenne avec 14 articles et 6—7 soies simples autour de l'écaille allongeé (fig. 3 D). Sur la hampe un gonflement extérieur serrate.

Labium cilié sur la lèvre ; lobes poilus avec 3 épines apicales fines (fig. 3 F). Maxille I à palpe finissant par 5 setae pennées, qui s'allongent vers son apex (fig. 3 G). Maxillipède à 2 épines sur le carpe et 3 rétinacles (fig. 3H). Mandibule à 2 fortes incisives brun rougeâtre, tout comme la face masticatrice ; 2 phanères richement ramifiés forment la lacinia mobilis ; palpe 3-articulé (fig. 4 F) avec le segment proximal a des soies longuement ciliées (flèche).

Chélipède à grand exopodite (fig. 3 I) et un seule épine sur le basispodite. Le bord préhensile des doigts n'a pas de tubercules, mais

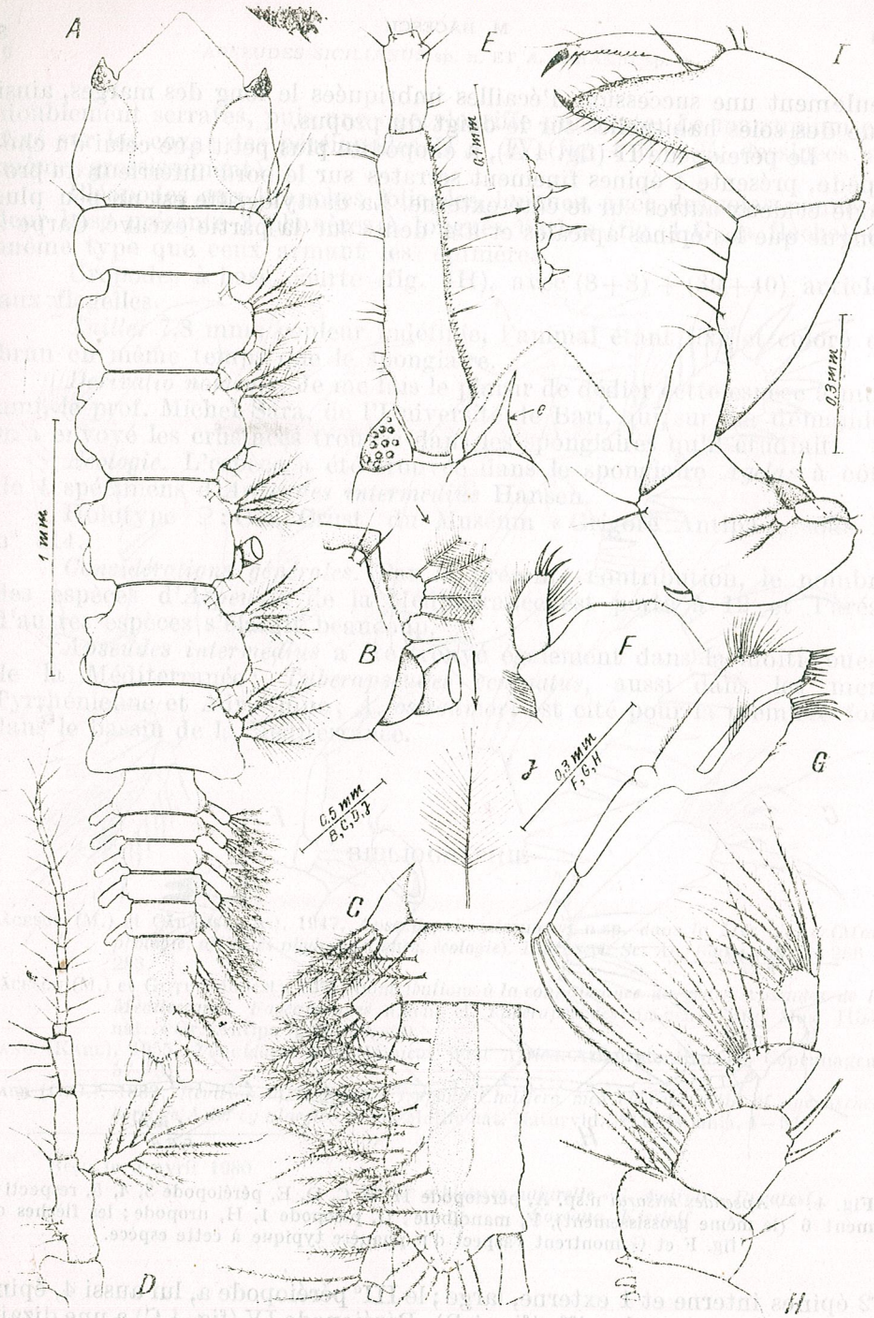


Fig. 3. — *Apseudes misarai* n.sp. ♀. A, vue dorsale ; B, bord du péréionite 4, agrandie ; la flèche = les soies penées ; C, extrémité de l'abdomen, agrandie, D, antenne ; E, partie frontale vue ventrale ; e, épine epistomale ; la flèche indique l'aspect serrate de l'article basal ; F, labium ; G, maxillule ; H, maxillipède II ; I, chélipède ; J, une seta du type spéciale de ces espèce, agrandie.

seulement une succession d'écailles imbriquées le long des marges, ainsi que des soies habituelles sur le doigt du propus.

Le péreiopode II (fig. 4 A), à exopodite plus petit que celui du chélipède, présente 4 épines finement serrates sur le bord inférrieur du propode et deux autres sur le côté externe. La dactylogriffe est un peu plus longue que les épines apicales et a 4 dents sur la partie excavé. Carpe à


Fig. 4. — *Apseudes misarai* n.sp. A, péréiopode II. B, C, D, E, péréiopode 3, 4, 5, respectivement 6 (le même grossissement), F, mandibule ; G, pléopode 1, H, uropode ; les flèches des fig. F et G montrent l'aspect d'u phanère typique à cette espèce.

2 épines interne et 1 externe, large ; le III$^{e}$ péréiopode a, lui aussi 4, épines et une longue dactylogriffe (fig. 4 B). Péréiopode IV (fig. 4 C) a une dizaine de griffes en faucille et un propus trois fois plus long que large, comme les autres d'ailleurs ; le V$^{e}$ Péréiopode a une faible rangée de lamines courtes (fig. 3 D) au bout du propode, tandis que le dernier péréiopode (fig. 4 E) est richement garni de lamines courtes et 3 épines longues et fines,



doublement serrates, puis une dactylogriffe puissante. Le marsupium est fixé sur les coxas des péréiopodes II à IV (fig. 4 B — E, dessinées au même grossissement).

Pléopodes aux branches foliacées, longues, avec des soies pennées ; leur base présente 3 phanères à longues barbes (fig. 4 G, la flèche), de même type que ceux armant les épimères.

Uropodes à base courte (fig. 4H), avec (8+8) + (39+40) articles aux flagelles.

*Taille.* 7,8 mm ; couleur indéfinie, l'animal étant fixé et coloré en brun en même temps que le spongiaire.

*Derivatio nominis.* Je me fais le plaisir de dédier cette espèce à mon ami, le prof. Michel Sara, de l'Université de Bari, qui, sur ma démande, m'a envoyé les crustacés trouvés dans les spongiaires qu'il étudiait.

*Ecologie.* L'espèce a été trouvée dans le spongiaire *Agelas* à côté de 4 spécimens d'*Apseudes intermedius* Hansen.

Holotype ♀ : Col. Crust. du Muséum « Grigore Antipa », sous le n° 514.

*Considérations générales.* Par la présente contribution, le nombre des espèces d'*Aspeudes* de la Méditerranée est porté à 12 et l'aréal d'autres espèces s'élargit beaucoup.

*Apseudes intermedius* a été trouvé également dans la moitié ouest de la Méditerranée ; *Tuberapseudes echinatus*, aussi dans les mers Tyrrhénienne et Adriatique ; *A. ostroumovi* est cité pour la première fois dans le bassin de la Méditerranée.

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# REMARKS ON THE GENERA *SCAPHIODONICHTHYS*, *BARBICHTHYS* AND *COSMOCHEILUS (PISCES, CYPRINIDAE)*

BY

PETRU M. BĂNĂRESCU

The genus *Scaphiodonichthys* differs from *Onychostoma* above all in the lower position of the lateral line and divergent striae on scales; its two species differ from each other in the number of teeth rows. Two subspecies can be recognized within *Barbichthys laevis*: *nitidus* in the South-East Asian mainland, *laevis* in Indonesia. The two species of *Cosmocheilus* differ mainly in the shape of papillae.

The three genera dealt with belong to the fauna of the Souh-East Asia (the Mekong and Menam Chao Phya river basins, the Malay Peninsula, the Kalimantan, Sumatera and Djawa islands); they include each one or two species.

*Material*: the specimens this study is based on belong to the following collections: The Academy of Natural Sciences in Philadelphia (A.N.S.P.), Muséum National d'Histoire Naturelle, Paris (M.N.H.N.), Rijks,museum van Natuurlijke Historie, Leiden (R.M.N.H.), the United States National Museum, Washington (U.S.N.M.), Zoölogisch Museum, Amsterdam (Z.M.A.).

***Genus Scaphiodonichthys*** Vinciguerra, 1890.

(= ***Scaphiodontopsis*** Fowler, 1934)

Vinciguerra [7] gives a good diagnosis of this genus, pointing out that it differs from *Capoeta*, *Scaphiodon* (presently considered a synonym of the previous genus) and *Dillonia* in having the pharyngeal teeth on two, not on three rows. In describing *Scaphiodontopsis* from the Menam R. basin, Fowler (4) does not make any reference to Vinciguerra's genus from the neighbouring Salwin R. basin in Burma, but compares it only with the West Asian *Scaphiodon* (a synonym for *Capoeta*) mentioning as differential characters the absence of barbels and low lateral line. Smith [6] synonymizes *Scaphiodontopsis* for *Scaphiodonichthys*, without discussing the value of their main differential character, the number of teeth rows.

The genus *Scaphiodonichthys*, in Smith's broader sense is characterized above all by its inferior, transversal mouth, almost total absence of the lower lip, that is present only at the corner of the mouth. By these characters, the genus can be included within the composite Varicorhinus Rüppell, 1835, as accepted by earlier authors (even in some very recent

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works, e.g. Wu [9]. Also, a few other characters of *Scaphiodonichthys* (absence of barbels, horny cover of the lower jaw, serrated dorsal spine, to a certain extent even the rather high number of branched dorsal rays) occur, in various combinations, in some other "*Varicorhinus*". Presently, *Varicorhinus* is no longer considered a valid, monophylletic genus ; its West Asian species are ascribed to *Capoeta* (syn. *Scaphiodon*) [5], the East Asian to *Onychostoma* with *Scaphestes* as subgenus [1], [2] and even the African Varicorhini are considered as belonging to several phylletical lines that evolved independently of the various groups of Barbus [1]. There are no "*Varicorhinus*" in India, nor in Indonesia ; of the three groups including "Varicorhini", *Onychostoma* ranges closest to Indochina where *Scaphiodontichthys* lives. In his recent revision of Chinese barbs, Wu [9] recognizes *Varicorhinus* as a valid genus, with two subgenera in the area : *Onychostoma* and *Scaphestes*. Fowler's *acanthopterus* being listed as a species of the first subgenus.

Actually, both *burmanicus* and *acanthopterus* share two important characters, one of which occurs neither in *Onychostoma* s. lato, nor in *Capoeta*, while the second is shared only with the geographically distant *Capoeta*, not with the neighbouring *Onychostoma* :

1. The position of the lateral line, that runs low on the caudal peduncle closer to the ventral than to the dorsal face. The number of circumpeduncular scales is, therefore, higher above than below the lateral line : 3 1/2 as against 2 1/2, on each side the total number being 14. In both *Onychostoma* and *Capoeta* the number of circumpeduncular scales is the same above and below the lateral line scale. According to the author's knowledge, there is no other genus of barbs in which the lateral line runs low on the caudal peduncle, this character occurs in several genera usually ascribed to the subfamily Rasborinae (= Danioninae) and in some of the Cultrinae.

2. The divergent disposition of the striae on the scales (Fig. 5). *Scaphiodontichthys* shares this character with the European and West Asian genera *Barbus*, *Capoeta*, *Aulopyge*, with the High Asian Schizothoracini [6] and with few other East- and South Asian genera, while in *Onychostoma* the striae are parallel, as in *Tor* and *Labeo*. It is remarkable in this respect that most genera with numerous divergent striae have a rather northern range, those with numerous and parallel striae, a more southern one ; yet the southern *Scaphiodonichthys* resembles, by this character, the northern genera, and the more northern *Onychostoma* reminds the southern group.

I think that these two characters justify the generical independence of *Scaphiodonichthys* (also including *Scaphiodontopsis*). These two characters seem to be more conservative than the number of teeth rows that differ in the two species, *acanthopterus* and *burmanicus* : the reduction of the number of rows is a derived character that occurred independently in various lines of cyprinids.

A more thorough study of many other characters is necessary in order to decide with which genus or group of genera is *Scaphiodonichthys*

[1] Dr. K. Bannister, *in litt.*



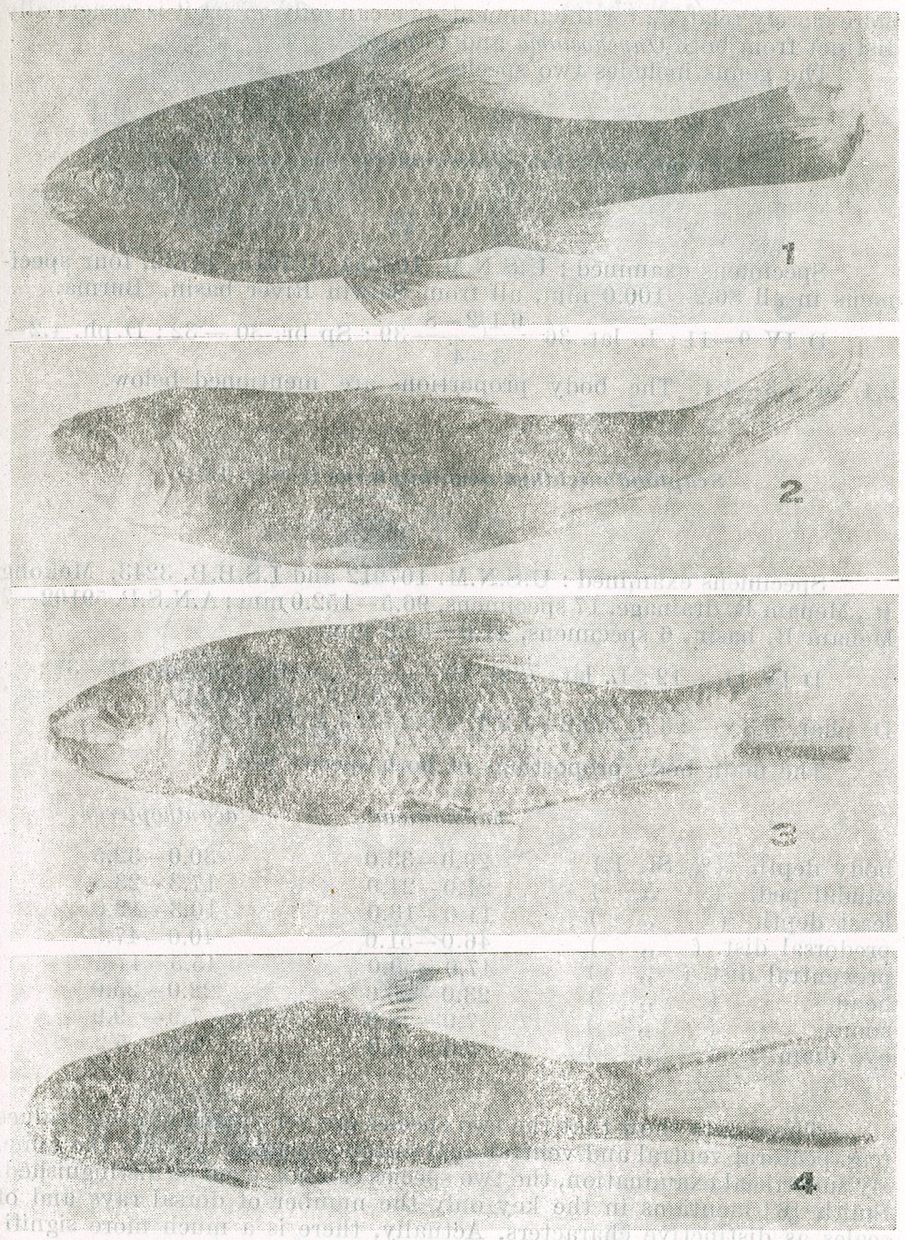



Fig. 1. — *Scaphiodonichthys acanthopterus*, male. U.S.N.M. 107912.
Fig. 2. — *Scaphiodonichthys burmanicus*. U.S.N.M. 103254.
Fig. 3. — *Barbichthys laevis nitidus*, lectotype. M.N.H.N. 3848.
Fig. 4. — *Barbichthys laevis laevis* Z.M.A. 115921.

more closely related ; for the moment, one can only assert it is generically distinct from both *Onychostoma* and *Capoeta*.

The genus includes two species :

***Scaphiodonichthys burmanicus*** Vinciguerra, 1890

Fig. 2

Specimens examined : U.S.N.M. 103254, 107915, 44732, four specimens in all 86.2—100.0 mm, all from Salwin River basin, Burma.

D IV 9—11 ; L. lat. 36 $\frac{6\,1/2-8}{3-4}$ 39 : Sp br. 30 — 32 ; D.ph. 4.2—2.4, or 4.3—3.4. The body proportions are mentioned below.

***Scaphiodonichthys acanthopterus*** (Fowler, 1934)

(Fig. 1)

Specimens examined : U.S.N.M. 107912 and I.S.B.B. 3243, Mekong R., Menam R. drainage, 17 specimens, 96.5—152.0 mm ; A.N.S.P. 59102—7 Menam R. basin, 6 specimens, 41.0—55.0 mm.

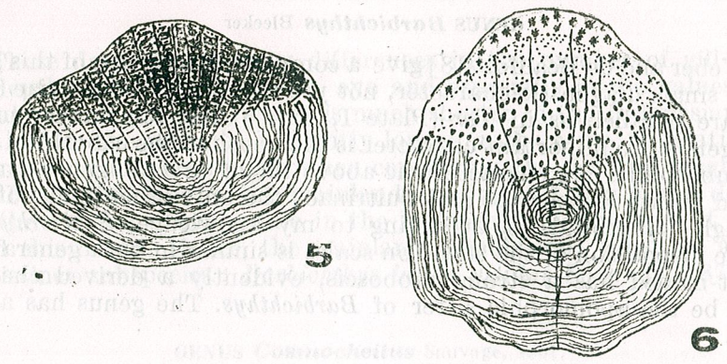
D IV 11 — 12 ; L. lat. (39) 40 $\frac{8-9}{3-3\,1/2}$ 42 ; Sp. br. 27—31 ; D. phar. 5.3.2—2.3.5, 5.3.2—1.3.4, 4.3.2—2.3.4, etc.

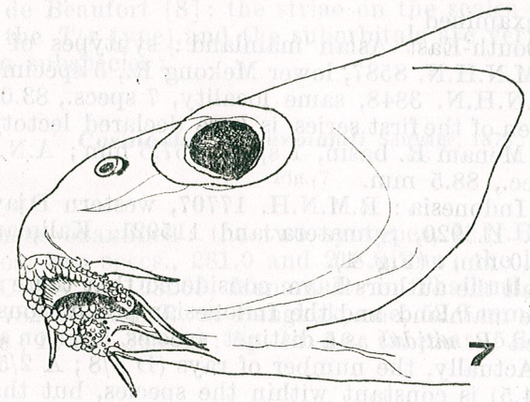
The main body proportions of both species are :

| | *burmanicus* | *acanthopterus* |
|---|---|---|
| body depth (% St. 1.) | 29.0—33.0 | 30.0—32.5 |
| caudal ped. ( ,, ) | 21.0—24.0 | 17.3—23.5 |
| least depth ( ,, ) | 11.0—13.0 | 10.3—12.0 |
| predorsal dist. ( ,, ) | 46.0—51.0 | 40.0—47.5 |
| preventral dist. ( ,, ) | 47.0—50.0 | 45.5—47.5 |
| head ( ,, ) | 23.0—26.0 | 22.0—25.0 |
| snout ( ,, ) | 7.0— 8.0 | 7.9— 9.9 |
| eye diam. ( ,, ) | 5.0— 6.0 | 4.3— 5.2 |

These data show that the two species are very similar ; other values (e.g. pectoral-ventral and ventral-anal distances) are practically the same. By superficial examination, the two species can not even be distinguished. Smith [6] mentions in the key only the number of dorsal rays and of scales as distinctive characters. Actually, there is a much more significant difference : the number of teeth rows ; this even justifies the separation of *acanthopterus* in a distinct subgenus, *Scaphiodontopsis* Fowler ; yet, since the genus has only two species, it is unecessary to recognize nominal subgenera.





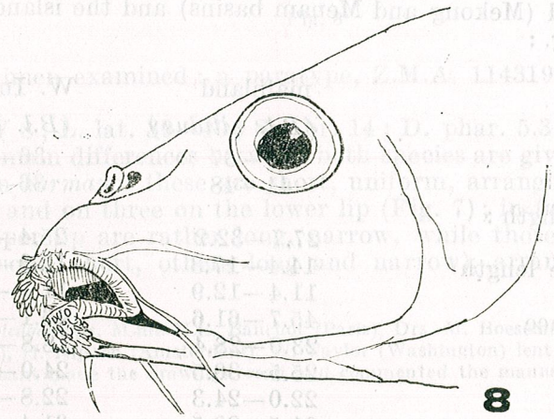




Fig. 5. — *Scaphiodonichthys acanthopterus*, scale.
Fig. 6. — *Barbichthys laevis*, scales.
Fig. 7. — *Cosmocheilus harmandi*, lateral view of head.
Fig. 8. — *Cosmocheilus falcifer*, lateral view of head.

### GENUS *Barbichthys* Bleeker

Weber and de Beaufort [8] give a complete description of this genus; I add a single diagnostic character, not yet mentioned: the striae on the scales are parallel, as in *Tor* (Plate I, Fig. 6) but less numerous than in this genus. An important character is the depth of the suborbital bones. High suborbitals are characteristic above all of several genera, usually included within Rasborinae and Cultrinae; the only other genus of barbs with high suborbitals is, according to my knowledge, *Schizorhynchus*; also, the disposition of the striae on scales is similar in both genera. Since the last-named has a strong proboscis, evidently a derived character, it may be the apomorphic sister of *Barbichthys*. The genus has a single species.

#### *Barbichthys laevis* (Valenciennes (1842)

Specimens examined:

From the South-East Asian mainland: syntypes of *Barbichthys nitidus* Sauvage, M.N.H.N. 8587, lower Mekong R., 5 specimens, 137.0—179.0 mm and M.N.H.N. 3848, same locality, 7 specs., 83.0—91.5 mm; the largest specimen of the first series is here declared lectotype (Fig. 3). U.S.N.M. 103288, Menam R. basin, 1 spec., 157.5 mm; A.N.S.P. 87245, same basin, 1 spec., 88.5 mm.

From West Indonesia: R.M.N.H. 17707, western Djawa, 1 spec., 95.0 mm; Z.M.A. 115920, Sumatera and 115921, Kalimantan island, 6 specs., 50.0—70.0 mm: (Fig. 4).

Up to now, all the authors have considered that there are no differences between the mainland and the Indonesian populations; even Sauvage, who described *B. nitidus* as a distinct species, later on synonymized it for *laevis* [3]. Actually, the number of rays (D 4/8; A 2/5) and teeth (D. ph. 5.4.2.—2.4.5) is constant within the species, but that of scales, gill rakers and several body proportions are different in the populations from the mainland (Mekong and Menam basins) and the islands of western Indonesia, e.g.:

| | mainland (*B.l. nitidus*) | W. Indonesia (*B.l. laevis*) |
|---|---|---|
| L. lat. | 36—37 | 36—38 (39) |
| Sp. br. | 41—48 | 36—45 |
| in % standard length: | | |
| body depth | 27.7—32.2 | 25.4—29.2 |
| caudal peduncle length | 14.4—17.3 | 12.9—16.9 |
| least depth | 11.4—12.9 | 11.1—12.3 |
| predorsal distance | 45.7—61.6 | 46.1—55.7 |
| P—V distance | 23.0—28.4 | 18.8—23.0 |
| V—A distance | 25.5—30.5 | 24.0—27.1 |
| pectoral length | 22.0—24.3 | 22.8—25.9 |
| head length | 21.5—28.5 | 31.4—34.0 |
| snout length | 9.7—12.0 | 12.8—14.0 |
| eye diameter | 5.0— 6.9 | 7.1— 8.9 |
| P in % of P—V distance | 84.0—99.0 | 100.0—123.0 |



These data clearly show differences in the number of gill rakers, body depth, head length, snout, eye and especially in the values of the pectoral-ventral distance, which is much shorter in the Indonesian populations; since the pectoral is slightly longer in the same populations, the differences are much stronger when comparing the pectoral length in % of the P—V-distance; in the mainland specimens the pectoral does not reach the ventral origin, while in the Indonesian populations it reaches beyond this origin. Hence, the mainland populations must be ascribed to a distinct subspecies: *Barbichthys laevis nitidus* Sauvage, 1878.

### GENUS *Cosmocheilus* Sauvage, 1881

Two generical characters have to be added to those mentioned by Weber and de Beaufort [8]: the striae on the scales are numerous and parallel (of the *Tor* type) and the suborbitals are very narrow, scarcely visible. Two subspecies:

#### *Cosmocheilus harmandi* Sauvage, 1878

Fig. 7

Specimens examined: the two syntypes, M.N.H.N. 9555 Mekong basin in Laos, two specs., 281.0 and 218.0 mm; the largest is declared lectotype: U.S.N.M. 103265, Menam R. in Thailand, one, 140.0 mm; A.N.S.P. 61782, Menam R. at Bangkok, one, 52.0 mm.

D IV 8; L. lat. 36—38; Sp. br. 16; D. phar. 5.3.2—2.3.5.

#### *Cosmocheilus falcifer* Regan, 1906

Fig. 8

Specimen examined: a paratype, Z.M.A. 114319, Sarawak., 139.5 mm.

D IV 8; L. lat. 39—40; Sp. br. 14; D. phar. 5.3.2.—2.3.5.

The main differences between both species are given by the papillae on lips: in *harmandi* these are short, uniform, arranged in two rows on the upper and on three on the lower lip (Fig. 7); in *falcifer* the papillae on the upper lip are rather long, narrow, while those on the lower lip unequal (some short, others long and narrow), arranged on four rows Fig. 8).

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# A CRITICAL VIEW ON DARWIN'S THEORY OF SEXUAL SELECTION

BY

DIMITRIE RADU

Analysing Darwin's theory of sexual selection, which attributes the secondary sexual characters in males, e.g. size, melodious voice, bright plumage, etc. to female preferences in choosing the mate, the author of this study refutes it as erroneous, grounded in anthropomorphic, subjective and sentimental arguments.

If Darwin's explanations fit the dimorphic species, they however fail to account for the frequent incidence of non-dimorphic species in which both sexes have either bright or dull feathers, or lack a melodious voice, or are of the same size. Moreover, Darwin's theory is totally infirmed by polyandrous species in which secondary sexual characters are reversed. All cases of sexual dimorphism to the advantage of males or females (in polyandrous species with the latter) are shown in this paper to be the result of two essential factors in the life of the species, acting in the period of reproduction viz, 1. the uneven sex-ratio between reproductive individuals and 2. the much larger number of reproductives than environmental ecological opportunities. The isolated or combined action of these factors leads to fierce competition between supernumerary individuals in order to eliminate mating competitors in the first case, and to conquer a reproduction territory, which is too small to satisfy the demands of the whole population.

Darwin had formulated his theory of sexual selection more than one century ago in his work "The Descent of Man and Selection in Relation to Sex". Despite the criticism it had been met with right after its elaboration, this theory is largely accepted even today.

A precise knowledge of Darwin's approach to the problem of sexual selection is convincing of the erroneous groundwork of this theory and we may rightly wonder why it is still being favoured nowadays.

Although the huge factic material Darwin had used in working out his theory of sexual selection is extremely valuable, his interpretation does not touch upon the real causes that have generated this phenomenon. But, we must remember the stage of biological development and anthropomorphic thinking of the time.

In the absence of the data subsequently supplied by ecology, Darwin's argumentation of his theory was based on criteria of a rather approximate scientific value. He considered for instance, that different male characters, e.g. bright plumage, melodious voice, force and combativity, etc. are engendered by the female taste of the respective species. Since specimens possessing such striking qualities seemed to be preferred, in time they accumulated in the respective males [1].

[1] To exemplify this interpretation I shall quote a paragraph indicative of the value attributed to sexual selection: "... preference is a phenomenon largely encountered also in nonvertebrates. In a population of *Drosophila melanogaster* experimentally formed of fifty

Thus, Darwin contends that : "To suppose that the females do not appreciate the beauty of the males is to admit that their splendid decorations, all their pomp and display are useless ; and this is incredible" (Chap. XVI, p. 496).

Darwinistic arguments in support of sexual selection are weak and contradictory, let alone their exaggerated anthropomorphism.

If we referred only to those species in which males are much more gifted [2] than females, Darwin's theory appears plausible and applicable. It, however, seems groundless when account is taken of the vast number of species in which both sexes have bright, or on the contrary, dull feathers alike to the environment they live in ; or have a flat voice. Darwin scarcely makes any mention of the existence of polyandrous species whose secondary sexual characters are reversed i.e. females are brighter, larger, have a stronger voice and represent the fighting sex ; he gives no explanation of this interesting phenomenon which refutes his whole theory of sexual selection (s.n.) [3].

Let us first examine some anthropomorphic affirmations made by Darwin in his theory of sexual selection. Speaking of males' attractiveness in keeping with female tastes, he says : "As any fleeting fashion in dress comes to be admired by man, *so with birds* a change of almost any kind in the structure or colouring of feathers in male appears to have been admired by females" (Cap. XIII, p. 385).

When males display their plumage and perform the nuptial dances Darwin opines that : "*they know what they are about and consciously exert their mental and bodily powers*" (Cap. VIII, p. 211). And furthermore, of all animals "birds appear to be the most aesthetic, ... excepting of course man, and they have nearly the same taste for the beautiful as we have" (Cap. XIII, p. 359). Also, males reveal their lively coloured parts "*to display their charms before the females*" (Chap. XIII, p. 363), and : "males are generally decorated with various ornaments ... *which are sedulously displayed before the females*" (Chap. XIII, p. 367). As for their songs these "*serve as a charm*, or merely as a call-note to the other sex" (Chap. XIII, p. 368).

Speaking of species like *Ardea* and *Buphus*, Darwin says that they have suffered changes in the course of time in accordance with "*change of fashion*," (Chap. XVI, p. 495) that is "of novelty having been admired

per cent wild forms and fifty per cent white-eyed mutants, it has been found, after twenty-five generations, that the mutant is practically eliminated by the fact that females from both categories show an exclusive *preference* (here it is !) for normal males to be reproductive mates" (M. Tufescu, 1976, p. 297). Other authors admit of sexual selection in the Darwinistic sense even in Jurassic and Cretaceous ammonites, and in extinct and living gastropods (Davitashvili, 1961, p. 480).

[2] In what follows we shall dwell upon examples from the world of birds, which are better known and furnish most typical cases of applicability of the theory of sexual selection ; Darwin himself devoting to it the largest part of his work (70 per cent).

[3] That the staunch defenders of Darwin's theory of sexual selection eschew an explanation of the mechanism of polyandry is seen also in their phrasing ; thus, "the fight *usually* takes place among males" (p. 8) ; "*in general*, males are more aggressive" (p. 371) ; "it is the males that *nearily* always are fighting" (p. 372) ; "fighting among the individuals of a sex (in the *vast majority* of cases, among males)" (Davitashvili, 1961,p.498). Neither do other works account for the origin of polyandry, nor is this phenomenon mentioned even (Botnariuc, 1967, 1976, 1979).



by birds for is own sake", (Chap. XV, p. 495) and further on, referring to birds' tastes, he states that : "It depends much on habit, as we see in mankind ; we may infer that this would hold good with birds and other animals" (Chap. XVI, p. 497). Even when treating the problem of differentiation among subspecies Darwin contends that the species migrating to new parts is faced with new conditions and : ' in this case sexual selection, which depends on an element liable to change — *the taste and admiration of the female* ... (Chap. XVI, p. 469).

Coming back to birds Darwin says that : "Whilst preening their feathers, they have frequent opportunities *for admiring themselves*, and of studying how best to exhibit their beauty". And : "that actions, *at first perhaps intentional*, have become instinctive ..." Furthermore, there is again anthropomorphism and contradiction : "If so, *we ought not to accuse birds of conscious vanity* ; yet when we see a peacock strutting about, with expanded and quivering tail-feathers, *he seems the very emblem of pride and vanity*" (Chap. XIII, p. 402).

In other places the above affirmations are again contradicted. Thus, trying to give an explanation to the preference shown by the females of some species for sound ranging from melody to shrieks and hissings, Darwin says : "But we must not judge of the taste of distinct species by a uniform standard ; *nor must we judge by the standard of man's taste*" (p. 309).

He then resumes an anthropomorphic formulation contradicting, at the same time, the above affirmation : "Even with man, we should remember *what discordant noises*, the beating of tomtoms and the shrill notes of reeds, *please the ears of savages*" (Chap. XVIII, p. 380).

Next come a series of like considerations : "Nevertheless it must be owned that the males of several brilliantly coloured birds have had their feathers especially modified for the sake of producing instrumental music, though the beauty of this *cannot be compared*, at least according to our taste, with that of the vocal music of many songsters "(Chap. XIII, p. 401).

Speaking of some sexual characters specific to males, which had been selected by the taste of their females and which, by our tastes, seem unattractive, e.g. the dull crest of the condor or the protuberance at the basis of the Chinese goose beak, Darwin says : "but we ought to be cautions in assuming that knobs and various flashy appendages *cannot be attractive to the female*, when we remember that *with savage races of man various hideous deformities* — deep scars on the face with the flesh raised into protuberances, the septum of the nose pierced by sticks or bones, holes in the ears and lips stretched widely open — are all admired as ornamental" (Chap. XIV, p. 426).

Here is what Darwin says about polyandrous species (Chap. VIII, p. 214) : "In the converse and much rarer case of the males selecting particular females, it is plain that those which were the most vigorous and had conquered others, *would have the freest choice* ; *and it is almost certain that they would select vigorous as well as attractive females*". And yet, on he goes contradicting himself : "if sexually-limited variations occurred in the females ... they would not be favoured or selected, *for the male usually accepts any female and does not select the more attrac-*

*tive individual*" (Chap. XV, p. 448). But, reconsidering the case, he adds : "It is however possible that the males may have selected the more attractive females"; only to affirm, a few lines further, that : "It is again possible that the females may have selected the more beautiful males, these males having reciprocally selected the more beautiful females". And he concludes : "*but it is doubtful whether this double process of selection would be likely to occur*, owing to the greater eagerness of one sex ..." (Chap. XVI, p. 482).

There is contradiction in the above assertions not only from one paragraph to the next, but also within one and the same sentence. In fact Darwin, obviously confused by his theory of sexual selection, says at a certain moment : "I may remark before proceeding that, under the present and next two classes of cases, the facts are so complex and the conclusions so doubtful, that any one who feels no especial interest in the subject had better pass them over" (Chap. XVI, p. 482).

Before proceeding to our view on the causes that have engendered sexual dimorphism in birds, let us examine the logic of Darwin's theory.

It strikes us from the very first that it hardly holds, because of the very mechanism on which it is grounded, namely, the selection by the females, which belong to the passive sex, of the males, which by their behaviour and characters represent the active sex, in such cases as this theory refers to. How could one admit logically that the male, bigger and more beautifully coloured, endowed with a stronger or more musical voice, executing the complicated nuptial dances, conquering a territory and defending it in battles with his rivals, therefore the male which stands for the fighting, a.o. does not choose his mate, but passively submits to being chosen? (Radu, 1970). It follows that he would accept any female that solicits him, be she unattractive for that matter, as Darwin himself acknowledges : "usually accepts any female, and does not select the more attractive individual" (Chap. XV, p. 448).

Trying to explain sexual dimorphism in some species to the advantage of males, on the basis of the would-be female selection in keeping with his theory, Darwin fails to produce a logical, scientific answer to the following situations : absence of sexual dimorphism ; absence of a stronger or more melodious voice in males ; occurrence of bright plumage, or of seasonal nuptial plumage in both sexes ; inversion of secondary sexual characters, a.o. He explains all these cases unconvincingly, or passes them over very lightly, ending up by recognising that : "No certain answer can be given to these questions" (Chap. XIV, p. 426).

As a matter of fact, Darwin, seemingly conscious of the relativity of his arguments uses now and again evasive, dubitative terms that express probability, and not certainty. He states : "that the female, though comparatively passive, generally exerts *some* choice and accepts one male in preferance to others" (Chap. VIII, p. 222). And he continues : "The exertion of some choice on the part of the female *seems* a law *almost* as general as the eagerness of the male" (Chap. VIII, p. 222). Or : "just as man can give beauty, according to his standard of taste, to his male poultry ... so *it appears* that female birds in a state of nature, have by a long selection of the more attractive males, added to their beauty



or other attractive qualities" (Chap. VIII, p. 211). And : "so with birds a change of almost any kind ... *appears* to have been admired by the female" (Chap. XIII, p. 385).

★

Analysing, in the light of present knowledge, the Darwinistic arguments that had led to the elaboration of the theory of sexual selection shows that the explanations given to the mechanism which generated this phenomenon do no longer hold.

True, in the Darwinist conception, the factors engendering sexual dimorphism are exclusively etologic, i.e. the simple "choice" [4] made by females constituting the foundation of the whole structure of the development of secondary sexual characters specific to the animal world, in general and to birds, in particular.

From a physiological and biochemical viewpoint, secondary sexual characters result from hormonal activity. The explanation of the appearance of the mechanisms of development of secondary sexual characters in the history of evolution has nothing in common with selection by one of the sexes, the female one, in general.

In our view, the biological phenomenon which Darwin presented under the name of sexual selection is the worke of a complex aggregate of ecological and etological factors whose interaction has generated, in various degrees, secondary sexual characters that are manifest in different ways e.g. colour, voice, behaviour, size, change of plumage, and express sexual dimorphism.

The analysis of sexual dimorphism in various species of birds shows a wide range of variations, from minor changes to actually fantastic differentiations between the two sexes [5]. We consider that this wide range of aspects and behaviours in both sexes of different bird species are the

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[4] Neither in my own observations on polygamous species, both in natural conditions and in captivity, nor in those of other investigators of the animal world have I ever found the female to choose a certain male during mating time. On the contrary, they assist with complete passivity and indifference to the complicated nuptial ritual of the males and even to their sometimes life-and-death fighting, to finally remain with the victor *regardless of which one he is*. In this case we cannot talk of the female "having chosen him".

[5] Besides the various ways in which it is manifest, sexual dimorphism differs also by the time interval in which it shows up. It can be permanent or appear strictly in the period of mating and growth of the young.

Sometimes, the bright colours of sexual dimorphism during the nuptial dances are masked by the bird's ordinary poise, being revealed at the right moment by certain movements of the feathers or of the body. Thus, in a clumsy typical steppe species like *Otis tarda*, a very original optical system has been developed i.e. the birds turn upwards the lower white side of their feathers, making thereby the white male visible from very long distances during the mating time.

In case also the females of some monogamous species have colours as bright as the males, without these being harmful to them, the signal of territorial demarcation appears to be twice as efficient since it exists in both spouses. While the male replaces the female in hatching, the latter takes his place in marking out the territory optically. Females have the same feather colours as the males only in the species that are nesting hidden in tree hollows or holes, when these characters are not dangerous for hatching. These are frequently encountered in the zoogeographical regions richest in bird species e.g. neotropical, Ethiopian, Indo-Malayesian where also the need for demarcation is much more acutely felt.

result of the interaction of two essential factors in their life, whose principal impact was felt during the most important phenological phasè of evolution i.e. during the period of reproduction.

These factors are :

1. The uneven sex-ratio between the representatives of the two sexes ;

2. The far greater number of reproductive individuals in comparison with the ecological opportunities offered by the environment.

The forms of monogamous, polygamous and polyandrous reproduction represent in fact the mode in which different bird species have resolved the problem of perpetuation in the conditions of the two imperatives : the uneven sex-ratio and the scarcity [6] of ecological opportunities for all individuals to reproduce themselves. In the period of reproduction these two factors stimulate among the individuals of the same sex, which participate in fecundation, a strong competition for winning the sexual partner and for securing the territory of reproduction.

Let us see now what were the consequences of this competition among the individuals of the same sex in point of the many transformations that have led to sexual dimorphism in different species of birds.

The monogamous species, comprising, in general, nidicolous birds, illustrate the need for both mates to participate in the optimal growth of their descendents. To this end, the male, which represents the active sex, is solicited to win its sexual mate and occupy a place for nesting likely to ensure the family's security and food throughout the period of growth of their young. This led to the active male's aggressive behaviour during the period of nesting and to the development of some optical, sonorous or physical means of warning and watching the territory of the nest against the greater number of individuals than nesting places. Thus, the males developed a series of characteristic features not because the females wanted them to be like that, as Darwin's sexual selection implies, but because they had to fulfil the imperatives for the existence of the species. These features helped them delimit the nesting territory and protect it against the trespassing of competitors, of conspecific bachelors which had been left *without nesting* places and even without mates (Radu, 1978). Environmental conditions have been the factors that decided on the modality of marking out the territory through the attributes developed by one sex, seldom by both (bright plumage, strong voice, etc.) so that the acquired characters could best serve the species. That would explain the different systems of demarcation used in different environments (optical, acoustic, behavioural), which the Darwinistic sexual selection attributes to the whims of the opposite sex. Thus, in afforested zones the territory is signaled out by vocal devices, in clearings it is the bright plumage, and in the vast open biotopes, like the steppe, the birds execute either short, vertical flights and sing, or soar high up in the air and sing, a.o. Aggressiveness and increase in size serve also to the defence of the territory and the winning over of the mate ; the latter easily accepts to couple with the "owner" of a

[6] The appearance of territorialism itself is a confirmation of this reality ensuing from the need to eliminate the supernumerary individuals of the same sex and from the scarcity of reproduction places for the whole population of the species.



territory protected from competitors, who provides food and security, having often already begun to build the nest, or even finished its construction.

The need for marking out the territory in accordance with the ecological ambient is very well illustrated by the birds that live in the vicinity of noisy, torrential waters since in such places acoustic demarcation would be ineffective and optical demarcation would be a handicap to birds staying on barren cliffs e.g. the water blackbird, the mountain wagtail, a.o. Although they belong to different orders, they have elaborated identical original modalities of marking out their territory, namely, repeated thrusting movements and, when standing stonestill, they dissemble in the rocky environment of cliffs. Wagtails, which live in the same noisy ambient, mark their territory by rapidly swinging their long tail. The mountain wagtail has a longer tail than the lowland species, because demarcation in these zones with waters, requires a more powerful system of signalization.

Sexual dimorphism with monogamous species thus appears to be the result of a "labour division" between the two sexes for the purpose of optimal reproduction of the species in the scarcity of ecological opportunities compared to the number of reproducing individuals.

Polygamous and polyandrous species include, in general, nidifugous birds whose young leave the nest right after eclosion, and therefore it is not necessary for both sexes to participate in the growth of their off-springs as is the case of nidicolous birds. Unlike monogamous species in which the development of sexual dimorphism was primarily required by the scarcity of reproduction places compared to the number of reproducers, in polygamous and polyandrous species these secondary sexual characters developed largely because of the exaggerated disproportion between the two sexes involved in reproduction i.e. the males outnumbering the females in polygamy-turned species and the other way round in the polyandry-turned ones.

This disproportion [7] between the reproducive sexes is an incontestable reality in the world of birds (Radu, 1970, 1979). Although the sex-

[7] In order to prove that there is an excedent of males and a limited number of nesting places, we shall quote some remarkable examples given by Darwin himself : "one of a pair of starlings *(Sturnus vulgaris)* was shot in the morning ; by noon a new mate was found ; this again was shot, but before night the pair was complete . . .". Another example : "During one season . . . he had shot thirty-five birds from the same nest ; these consisted of both males and females. Nevertheless it is a strange fact that within the same district, during the height of the breeding-season, there should be so many males and females always ready to repair the loss of a mated bird. Why do not such spare birds immediately pair together?" (Chap. XIV, p. 409 — 410).

But, instead of these interesting findings leading him into realizing the existence in nature of an overnumber of individuals and implicitly of the limited ecologic opportunities compared to the overall population of the species, Darwin answers to J. Weir who upholds that : "certain males and females do not succeed during the proper season in exciting each other's love and consequently do not pair?" Here is Darwin's retort : "This suspicion will appear somewhat less improbable after we have seen what strong antipathies and preferences female birds occasionally evince towards particular males" (Chap. XIV, p. 410). He, therefore, strongly supports the false explanation of females' "taste" for male justifying thereby the existence within one and the same population of so many pairless individuals : "that some males and females of the same species, inhabiting the same district, do not always please each other and consequently do not pair" (Chap. XIV, p. 407).

ratio at birth is relatively even, yet in the end we have a numerical discrepancy between the reproductive sexes, because of behavioural particularities, different resistance to diseases and adversities, different age of sexual maturity in the two sexes, the different impact of the birds of prey, a.o. which account for a different death-rate of each sex.

Polygamous species had a greater number of males. To win over the females, which were in lower number, males began to compete developing their secondary sexual characters with a view to acquiring some features or behaviours like the ones discussed previously. Thus, the fierce competition among males is responsible for the development of their secondary sexual characters which have the role of strong signals of warning the competitors or even of fighting among the individuals of the supernumerary sex [8].

In polyandrous species whose females outnumber the males, the phenomenon took place vice versa, namely competition among the former developed their secondary sexual characters, females acquiring thus the features of polygamous males.

We may therefore assess that the secondary sexual characters have appeared as a result of the interaction of two factors which govern the life of the species, namely, *the uneven number of the two reproducive sexes and the existence of limited reproduction opportunities compared to the total population*, even for the individuals of the numerical majority sex. Therefore secondary sexual characters have been produced by the competition between the individuals of the supernumerary sex associated with the limited number of places of reproduction, a fact that enhanced competition and stressed even more the manifestations of sexual dimorphism in the animal world.

## CONCLUSIONS

An analysis of Darwin's theory of sexual selection in the light of current knowledge proves it to be obsolete, because it has a strong anthropomorphic and subjective character. The existence of polyandrous species refutes the whole mechanism used by Darwin to explain the origin of sexual dimorphism in the animal world.

Sexual dimorphism in birds is, in our opinion, the result of the separate or combined action of two factors, with the prevalence of the effects of one or the other, as the case might be : a) The uneven number of the two sexes in the phase of reproduction ; and b) The limited reproduction opportunities for the whole population of the respective species.

Because of these factors a strong competition develops between the individuals of the supernumerary sex generated in the first case by the need to eliminate the competitors and to win over the sexual partner, which is in numerical minority, and in the second case, by the need to secure a territory for the breeding of the young.

[8] The importance of the competitive substratum as generating factor of sexual dimorphism is proved by fighting often taking place in the absence of females, males alone gathering on the battlefield to eliminate the weaker specimens.



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# NOTES ON THE LIFE HISTORY OF *BRACHYIULUS CALCIVAGUS**, A MILLIPEDE NEW TO EGYPT

BY

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Samples were taken to determine the course of post-embryonic development and life history of the millipede *Brachyiulus calcivagus* in the field. Stadial determination was possible from counting the podous and apodous segments, rows of ocelli and analysis of dimensions. Sexual differentiation appeared at stadium VI. Maturity is usually attained by males in the eighth stadium and by females in the ninth stadium. Both sexes proceed to the tenth stadium. Periodomorphosis or "intercalary" phase was observed in some male individuals of the ninth stadium. The significance of the life history characters was discussed.

## INTRODUCTION

The small dark brown millipede *Brachyiulus calcivagus* is found in underneath pots crowded together in plant nurseries and in thickly compacted and decayed plant leaves and other surface debris of the Botanic Gardens. In terms of overall density it would not reach such a high density as Polydesmids, *Orthomorpha gracilis* and the blaniulid *Blaniulus sp.*[1] which were found together. In Egypt nothing has been reported about the species. The object of this paper is to gather data about the field life history of the species. Details of the field life histories of various species of millipedes have been given in [1] — [13], [18] [19], [23]. These authors found that in some julid millipedes, females can moult several times after first attaining maturity but males cannot, yet in some species, particularly in members of the Schizophyllinae, adult males moult into an intercalary stage in which the secondary sexual characters regress towards an immature condition. In this paper I attempt to discover the field life history of the species and to determine whether the functional to non-functional male moult is the usual manner of development in *Brachyiulus calcivagus*.

## SAMPLING PROCEDURE

Six samples were taken from the Botanic Gardens of the Faculty of Science in May, July and September 1973; December 1978 and February 1979. Each sample consisted of 10 units and each unit consisted

* After K. Strasse, 1976 (Über Diplopoda- Chilognatha Griechenlands : II Rev. Suisse Zool. 83, 3, 579 — 645) the species studied in this paper would be a subspecies of *B. lusitanus* (i.e. *B. lusitanus calcivagus* Verh. 1910).

[1] This species is currently under taxonomic review by Mr. J.G. Blower.

of 0.04 m² of soil, including the surface litter, if present, to a depth of 10 cm. Each unit was extracted by Tullgren funnel.

## DETERMINATION OF THE STADIA

Stadia are characterized by the succession of segment numbers, by the probability method described in [1] and by the use of the ocular field method described in [21] and [20]. Collected animals were analysed by both these methods. A brief account of each method is given. Concerning the first method Blower and Gabbutt [1] suggested that the apodous segments of a given stadium are incorporated into the podous series of the succeeding stadium. For example, a second stadium with 6 podous and 5 apodous segments develops into a third stadium with 11 podous segments (see the entries in columns b and c of Table 1, against stadia II and III). This method proved satisfactory for the characterization of the early stadia, but by the time maturity is reached, the segment number of a given stadium begins to overlap those of the preceding stadium (see Table 1 for stadia VIII, IX and X). In this case the second and third methods have practical implication. The principle of the ocular method is evident from Fig. 1. A new row of ocelli is added to the ocular field at each moult. Since the first ocellus does not appear until the second stadium the stadium number is equal to the number of rows plus one. The probability method implies the measurement of the length and breadth of each individual in the entire collection. These measurements were then plotted by frequency groups on arithmetic probability paper [14] and sorted into groups of normally distributed lengths and breadths. Each group was then examined to determine whether the sum of the podous and apodous segments of its contained individuals predicted the range of podous segments in the next largest group. Finally the groups were validated as stadia by individual probability plots. Means and standard deviations either side of the mean were read-off directly

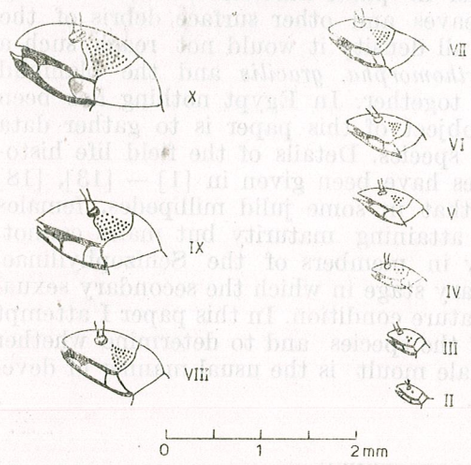


Fig. 1. — The ocular field of *Brachyiulus calcivagus*. The original ocellus of the second stadium forms the posterior apex of the triangular field (lowermost in the figures). The most recently added row of ocelli forms an antero-dorsal side of the field (uppermost in the figures).



from the probability plots. Lengths were determined by drawing each animal on graph paper using a squared graticule in the microscope eyepiece and the mid-lateral line from the head to the posterior edge of the anal valves was determined by map-measurer. Breadths were measured directly with a calibrated eyepiece in profile.

## ANAMORPHOSIS

Stadium I was not present in the samples. Information on this stadium has been obtained from eggs reared in vivaria. As in most other Julids the first stadium does not leave the adherent pupoid skin, nor does it leave the egg case. The second stadium is the first to walk out of the egg capsule and take up food and this is the first stadium to emerge from the sample units in the funnels. In some julidae, e.g. *Ophyiulus pilosus* and *Julus scandinavius*, the third stadium is the first to move around and take up food from the environment [1]. Figure 2 shows the succession of the stadia. Individuals of stadium VI first show the beginnings of the secondary sexual differentiation which is apparent by the loss of the two pairs of legs of the seventh segment. Mature males, which are morphologically distinct by the form of the first pair of legs that are hooked, first occur in stadium VIII and continue to moult into stadium X, but the intercalary stage (non-functional males) alternates with the mature (copulatory) stage (see below). Females of stadium VIII did not contain large or enlarging eggs while individuals of stadium IX and X contained full sized eggs. Thus females attain a state of maturity later than males. This phenomenon has been observed in many other Julidae [7]. It appears that *Brachyiulus calcivagus* is passing through ten stadia. Data on segment numbers and increments are presented in Table 1. Table 1 also includes figures for intercalary males in the ninth stadium. An estimate of volume has been derived from the dimensions by putting $V = \pi r^2 l$, assuming the animals to be perfect cylinders. If the logarithm of this value is plotted against stadium, the slope of the straight line fitted to the points by eye is 0.315 and its antilogarithm is 2.065; thus the volume approximately doubles at each moult as was shown in [1], [5] for some British Julidae.

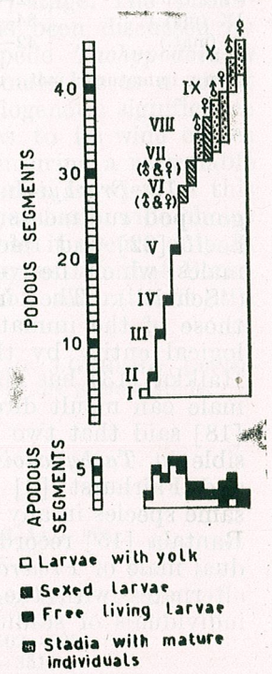


Fig. 2. — Anamorphosis in *Brachyiulus calcivagus*. The number of podous and apodous segments are shown for each of the stadia.

*Table 1*

Anamorphosis of *Brachyiulus calcivagus*: podous and apodous segments and dimensions of each stadium

| Stadium | Podous segments | Apodous segments | Mean ± S.E. sample size in parenthses | | Volume ($mm^3$) |
|---|---|---|---|---|---|
| | | | Length (mm) | Breadth (mm) | |
| I | 4 | 2 | | | |
| II | 6 | 5 | | | |
| III | 11 | 4 | 1.4±0.5 (10) | 0.25±0.01 (10) | 0.068 |
| IV | 15 | 6 | 2.5±0.6 (10) | 0.31±0.01 (10) | 0.188 |
| V | 21 | 5—6 | 4.3±0.4 (12) | 0.46±0.02 (12) | 0.714 |
| Males | | | | | |
| VI (imm.) | 27—28 | 2—3 | 6.5±0.8 (7) | 0.56±0.03 (7) | 1.601 |
| VII (imm.) | 29—31 | 2—3 | 8.7±0.7 (9) | 0.73±0.06 (9) | 3.642 |
| VIII (mat.) | 32—34 | 2 | 10.7±1.0 (16) | 0.78±0.05 (16) | 5.112 |
| IX (mat.) | 33—38 | 1—3 | 12.4±1.3 (15) | 0.83±0.07 (15) | 7.542 |
| IX (intercalary) | 34—37 | 1—3 | 12.1±1.1 (5) | 0.84±0.09 (5) | 6.706 |
| X (mat.) | 40—43 | 1—2 | 14.4±1.1 (12) | 1.0±0.05 (12) | 11.311 |
| Females | | | | | |
| VI (imm.) | 27—28 | 2—3 | 6.5±0.75 (6) | 0.58±0.04 (6) | 1.717 |
| VII (imm.) | 29—31 | 2—3 | 9.0±0.8 (11) | 0.79±0.04 (11) | 4.412 |
| VIII (imm.) | 32—36 | 2 | 11.3±1.1 (19) | 0.85±0.03 (19) | 6.26 |
| IX (mat.) | 34—39 | 2—4 | 14.8±1.3 (12) | 1.07±0.09 (12) | 11.62 |
| X (mat.) | 42—45 | 1—2 | 16.3±1.1 (15) | 1.1±0.08 (15) | 15.492 |

imm., immature; mat., mature

## PERIODOMORPHOSIS

In *Brachyiulus calcivagus* some males in the ninth stadium have gonopod rudiments. This phenomenon has first been described by Verhoeff [22] and referred to as "Periodomorphosis". The non-functional males which derived from mature males are called intercalary males ("Schalt"). The intercalary male has gonopod rudiments similar to those of the immature male, but is mainly characterized as a morphological entity by the form of the first pair of legs which are hooked. Halkka [13] has shown in *Schizophyllum sabulosum* that an intercalary male can moult directly into another intercalary male. Sahli [16], [17], [18] said that two or even three consecutive intercalary males are possible in *Tachypodoiulus albipes* (called *T. niger* in Britain), and Blower and Fairhurst [2] mentioned that intercalary males can occur in the same species in any of the later stadia from the ninth stadium upwards. Rantala [15] recorded eight successive intercalary phases in one individual male of *Proteroiulus fuscus*. In the present study an intercalary stage alternates with the mature ("copulatory") stage. This occurs in male individuals of stadium IX but not of stadium X.

## DISCUSSION

The number of immature stadia in the life history of *Brachyiulus calcivagus* is seven or eight. The significance of reducing the number of immature stadia and consequently the average duration of a generation



in a species of millipedes has been discussed by Blower and Gabbutt [1]. They have pointed out the advantage of earlier maturity in *Cylindroiulus latestriatus* and the subsequent elevation of its intrinsic rate of natural increase 'r', to enable the species to colonise new sites. The corollary that *C. latestriatus* can spread all over the world has been stressed by Blower [4]. Although little is known about the continental distribution of *Drachyiulus calcivagus*, Blower (personal communication) informed me that the species can be found in North America. In this case, the contraction of the life history of *Brachyiulus calcivagus*, i.e. the shortening of the time taken to mature may confer great ecological advantage.

In some species of Julidae, e.g. *Cylindroiulus punctatus* and *Cylindroiulus latestriatus*, males do not appear to moult after attaining maturity for the first time, but females are considered to moult in three or more successive years [1]. In the Julines, *Julus scandinavius* and *Ophyiulus pilosus*, neither males nor females moult after achieving maturity [5], [6]. In *Brachyiulus calcivagus*, males and females moult after achieving maturity for the first time, whether females are able to breed in each of the adult stadia is not clear at the present; but in males, the intercalary stage alternates with the mature (copulatory) stage. The phenomenon of Periodomorphosis or intercalary stage has been discussed by Blower and Fairhurst [2] in the case of the millipede *Tachypodoiulus niger*. They suggested that the extension of adult male life as a result of Periodomorphosis is of ecological rather than phylogenetic significance as postulated by Verhoeff [23] — adapting the species to its wide choice of habitat (eurytopic character of the species) and ensuring a reasonable sex ratio in those areas where the species is least dense. In view of this statement we may conclude that *Brachyiulus calcivagus* has a wide choice of habitat, but from the surveys made, it results that it occurs only in the sheltered houses of the Botanic Gardens. The suggestion that Periodomorphosis is an adaptation to maintain a reasonable sex ratio is still valid and needs further investigation.

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# CYTOCHEMICAL LOCALIZATION OF ADENYLATE CYCLASE IN FISH OVARY

BY

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Using the lead phosphate method we demonstrated the presence and the localization of adenylate cyclase in the fish ovary. In the follicular cells, the reaction product was widely distributed in association with plasma membranes, and smooth endoplasmic reticulum membranes. In addition, the enzyme activity was demonstrated in the endothelial cells (capillary endothelium of the ovary : plasma membranes, Golgi elements, mitochondria and in the nucleus. The cytochemical findings indicate the presence of adenylate cyclase in developted or mature fish oocytes : in membrana radiata, smooth membranes of endoplasmic reticulum, Golgi elements and at the level of yolk platelets.

Numerous investigations brought evidence on the participation of the adenylate cyclase system in the mechanisms by which the cells capture hormonal information 111, 121, 191. That is why it proves particularly important to identify adenylate cyclase receptors in the gonads, as well as in the course of their development and maturation.

Studies on the cytochemical localization of the adenylate cyclase in the gonads are scarce. Chakraborty et al. [3] identified adenylate cyclase in the plasma membrane and in the inner mitochondrial membranes of spermatides in mice. It was also described in the plasma membranes of luteal cells, in the rabbit ovary and in the corpora lutea in bovines [8], [10], [11], [13]. Adenylate cyclase was accepted as marker enzyme for plasma membrane, but its presence in some of the internal cellular membranes was not excluded [4]. Adenylate cyclase was not identified cytochemically in the ovary of lower vertebrates.

Although the importance of hypophyseal gonadotropic hormones in the growth and development of gonads is recognized, their action at cellular level is little known. Injections of gonadotropic hormones (FSH and LH) enhance the adenylate cyclase activity in the rat ovary and fish ovary (Carassius auratus) [7]. The particular mechanisms of action of FSH and LH on follicular cells and the capacity of cyclic nucleotide ($AMP_c$) formation in the prepuberal ovary in rat were also suggested [1], [6], [12].

Considering the importance of adenylate cyclase receptors for the hormonal regulation mechanisms of gonads, we carried out a study on the ultrastructural cytochemical localization of receptors in the ovary oocytes of teleost fish, in the course of their growth and maturation.

## MATERIAL AND METHODS

Our experiments were carried out on two fish species, namely: golden crucian *(Carassius auratus gibelio)* and Chinese carp *(Hypophthalmicthys molitrix)*. The animals were supplied by the piscicultural research station Nucet (Dîmbovița).

The ovary was removed, minced in small fragments and washed in saline solution of NaCl 0.6%. The tissues were fixed in a glutaraldehyde solution 2.5%, for 15 min and then washed four times successively with cold cacodylate buffer 0.1 M (pH 7,4), for 15 min in each bath. For cytochemical demonstration of adenylate cyclase the technique described by Cheng and Farquhar was used [4]. The incubation medium for the enzyme identification consisted of Tris maleate buffer 0.1 M (pH 7.4), theophylline 2 mM, sucrose 3%, magnesium acetate 4 mM, ATP 2 mM, NaF 15 mM and Pb acetate 2 mM, which was the last component to be introduced. Before using it, the solution was filtred to eliminate the precipitate. Incubation was carried out for 2 hours at 30°C. The reaction was stopped by washing the pieces in cold cacodylate buffer (0.1 M, pH 7.4). The tissues were postfixed in 1% osmic acid in acetate-Veronal buffer for 2 h at 4°C.

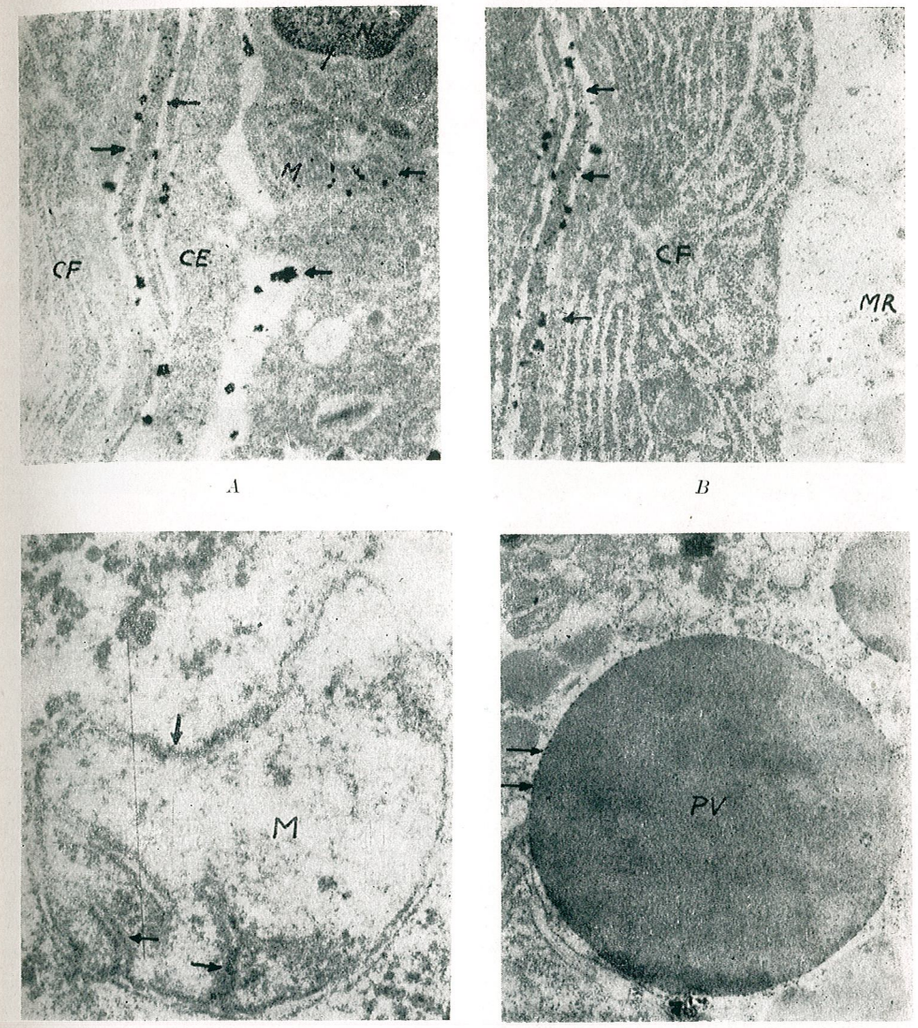
The pieces were dehydrated and embedded in Epon 812. The ultrafine sections were examined on an electron microscope Philips EM 201. The analysis of localization of adenylate cyclase (precipitate of Pb pyrophosphate) was done on non-stained sections or stained with an aqueous uranyl acetate solution 1%.

## RESULTS

As it is shown in Fig. B, Plate I, the presence of adenylate cyclase in the follicular cells of fish ovaries is obvious on sections not stained with uranyl acetate. The enzyme is localized in the plasma membrane of the follicular cells, topographically distributed on both sides, as well as in the membrane structure. It is cytochemically identified as black precipitates with a granular distribution (see arrows). Noteworthy is the fact that, in follicular cells, adenylate cyclase was also identified ultrastructurally in the membranes of some cisternal vesicles of the Golgi apparatus (on crucian ovary sections).

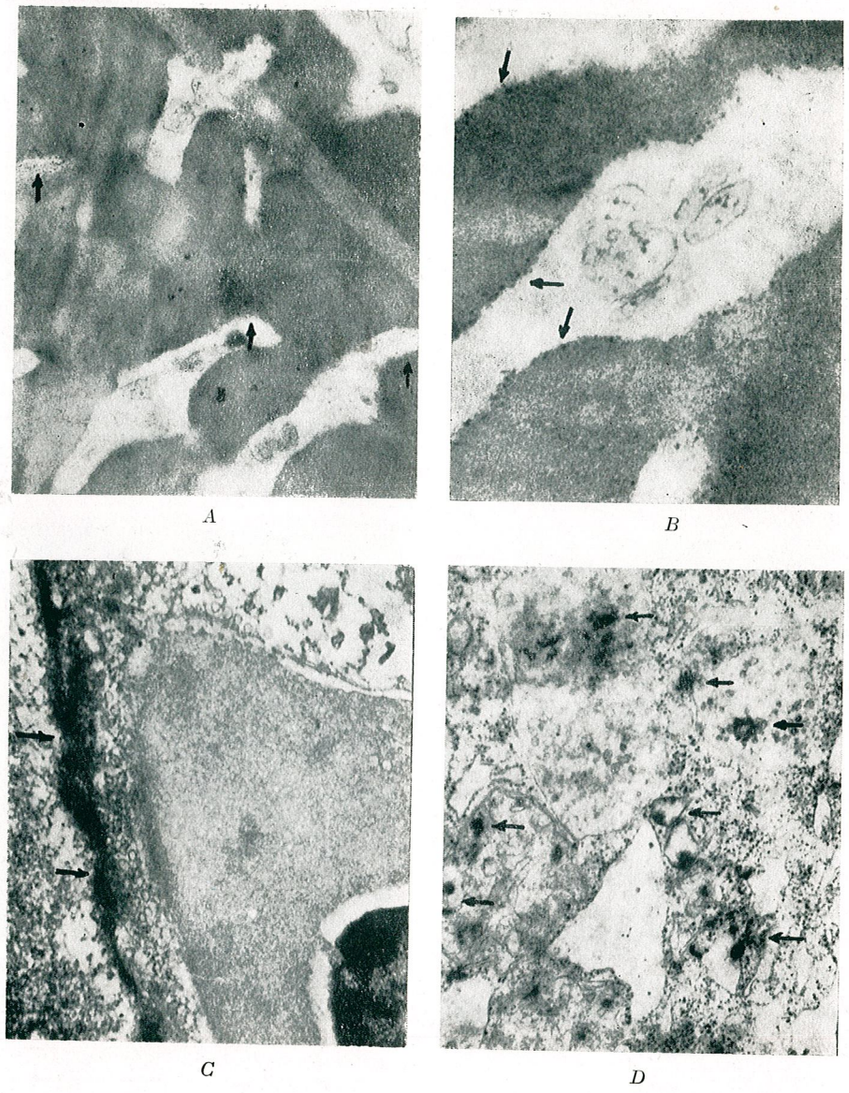
The adenylate cyclase reaction product could also be demonstrated on endothelial cells (capillary endothelium) of the fish ovary, under the form of abundant precipitates (Pl. II, Fig. C). Following up the intracellular distribution of the enzyme in endothelial cells, the presence of adenylate cyclase was also found on the Golgi elements membrane, in mitochondria and in the nucleus (Pl. I, Fig. A). The presence of adenylate cyclase in these structures confirms some biochemical observations reported in rat liver [4], [14].

In the plasma membrane of young oocyte cells (stage II — III), no adenylate cyclase receptors were identified cytochemically. In exchange, in large oocytes, in the area of microvilosities of membrana radiata, a finer granular precipitate was identified, indicating the presence of the




A B C D

Plate I. *A*) Capillary endothelium of the fish ovary *(Carassius auratus gibelio)*; fixation with aldehydes and $OsO_4$ but unstained with uranyl acetate. Note the localization of reaction product in plasma membrane, Golgi elements, mitochondria (M) and nucleus (N). CF-follicular cell; CE — endothelial cell. 7500 × ; *B*) Follicular cell of the fish ovary *(Carassius auratus gibelio)*. Section unstained with uranyl acetate. Lead phosphate deposits are seen in association with the plasma membrane (see arrows). CF — follicular cell; MR — membrana radiata. 7500 × ; *C*) Cross section of a mitochondria of the oocyte from Chinese carp *(Hypophthalmichthys molitrix)*. It can be seen that the deposits are present along the outer surface of mitochondrial membranes (see arrows). 10.500×. *D*) Cross section of a yolk platelet (PV). The lead phosphate deposits are located along the outer surfaces of yolk platelet and in the inner structure. 7500 × .



A B C D

Plate II. *A*) Cross section through the membrana radiata of fish ovocyte from *Carassius auratus gibelio*. Note the localization of reaction product in the microvilosities area (see arrows). 7500 × ; *B*) Higher magnification of microvilosities area of the same membrana radiata (70.000 × ). Note the localization of adenylate cyclase on the outer surface of the membrane ; *C*) Capillary endothelium of the fish ovary *(Hypophthalmichthys molitrix)*. The lead phosphate deposits are present along the membrane of endothelial cell (see arrows). 7500 × ; *D*) Section through the cytoplasmic area of the fish ovocyte from *Carassius auratus gibelio*. There are numerous deposits of lead phosphate on cytoplasmic membranes (see arrows). 10.500 × .



enzyme on this structure. (Pl. II, Fig. A, see arrows). At higher magnifications of microvilosities area of membrana radiata (70.000 × ) the distribution of these precipitates was very clear : the enzyme is topographically distributed on either side of the membrana radiata microvilosities (Pl. II, Fig. B, see arrows). This adenylate cyclase distribution was found in the membrana radiata in both fish species.

Apart from the localization in the membrana radiata area, the adenylate cyclase reaction product could also be demonstrated at the level of other fish oocyte structures. As shown in Pl. I, Fig. C and Pl. II, Fig. D, the lead phosphate deposits were found on the inner mitochondrial membranes (see arrows). The enzyme was also present at the level of some cytoplasmic membranes belonging to the vesicular system of the Golgi elements and the membranes of the smooth endoplasmic reticulum (Pl. II, fig. D.). In the case of cisternal elements, the reaction product was more concentrated on the inside of the Golgi membranes.

Following up the cytochemical localization of adenylate cyclase on sections through fish vitelline platelets, the presence of the reaction product on these structures was also found (P. I, Fig. D, see arrows). The lead phosphate deposits were identified at the periphery of yolk platelets, and occasionally observed over the central region (in its inner structure).

## DISCUSSION

Our cytochemical data point out a well individualized adenylate cyclase receptor system in the plasma membrane of follicular cells of teleost fish ovaries. The cytochemical results extend the biochemical findings obtained in the crucian [7] and particularly in mammals [10], [14]. The importance of the follicular cells in fish and other animals has been pointed out in numerous papers [1]. Pituitary ablation in fish disrupts the generation of primary spermatocytes, atresia of all eggs in which vitellogenesis is well advanced with the development of corpora atretica. The presence of adenylate cyclase receptors suggests that the gonadotropic hormones exert their regulating influence on oocytes' development through follicular cells.

In advanced development stages the membrana radiata of the oocyte is well individualized morphologically and also shows adenylate cyclase receptors. The elaboration of a hormonal reception apparatus in the membrane of oocytes enables the latter to free themselves from the cooperation with follicular cells. Rao et al. [12], studying the hormonal regulation in rat ovary, showed that ovarian follicular growth and differentiation are dependent on appropriate stimulation by estrogens and gonadotropins. They acted on prenatal follicles to stimulate proliferation of granulosa and theca cells. In the absence of hormonal stimuli, both cell types cease to proliferate and the follicles undergo atresia. In contrast, LH caused an irreversible cessation of proliferation on theca cells. These observations, together with ours, suggest that changes in proliferation can be correlated with specific hormone-induced morphological growth and changes in receptor content. Other authors suggest that LH and

FSH hormone stimulate particular adenylate cyclase in the ovary [6], or act on two distinct cell types, those of the granulosa and the theca [1].

The presence of adenylate cyclase on well-developed oocytes has multiple functional significations, particularly for a differentiating cell [2]. In piscicultural practice, it is well known that hypophyseal powders and preparations of gonadotropic hormones, shorten the maturation duration of fish gonads. We could assume that this process is achieved by a direct intervention of the gonadotropic hormone with adenylate cyclase receptors of developed oocytes. It was also suggested that LH favours the breakdown of germinal vesicle in meiotic maturation by enhaced LH-induced lysosome activity [5].

The presence of adenylate cyclase at the level of yolk platelets emphasizes the importance of cyclic AMP in the control of phosphorylation processes of the proteins stocked in these structures during morphogenesis. Menon and Azhar [10] have shown that administration of chorio-gonadotropin produced an increased concentration of cyclic AMP and stimulated the protein kinase activity in rat ovarian cells. The results provide evidence for a probable intracellular compartimentation of cyclic AMP in the ovarian cell, for regulating specific metabolic processes. Moreover, cyclic nucleotide significantly stimulated the activity of some rate-limiting enzymes in the glycolysis system of unfertilized sea urchin eggs [16] and following fertilization [15].

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# INCORPORATION OF 3H-THYMIDINE IN THE OOCYTES OF *CARASSUS AURATUS GIBELIO*

BY

M. IONESCU VARO

3,8.10,11,13

The use of tritiated thymidine as tracer of rDNA, which amplifies during oogenesis in *Carassus auratus*, reveals extended replication zones around diplotene free nucleoli occupying sometimes inner surfaces of labeled rDNA which comprise formative nucleoli. As the oocyte grows, the number of nucleoli grows to 1280, their volume becoming increasingly smaller when the vacuoles with arylesterase mucopolysaccharides and acid phosphatases occur. In this stage they are surrounded only by a few small labeled granules, after which replication ceases.

Radioisotopic studies of egg formation in fishes have been attempted only by Vincent [7], who has found that the oocytes of *Roccus saxatilis* have ten times as many ribosomal cistrons as erythrocytes, and by Vlad [9], who opines for top lebeling in the pachytene stage in *Salmo irideus*, in which the synthetized amount per oocyte is of 20 μg DNA.

The present paper has been aimed at the real topography of tritriated thymidine labeling during oogenesis in *Carassus auratus gibelio*.

## MATERIAL AND METHOD

Freshly excised ovaries were incubated for 6 hrs in a sterile substrate consisting of 10 ml fish culture medium (NaCl 0.78, KCl 0.066, $SO_4K_2$ 0.068 g/100 ml), 6 ml water, 0.03 g/ml crystalline bovine plasma albumin, and 50 μl (50 μc)/ml of a solution of [3H] thymidine (1.9 c/mM) supplied by The Radiochemical Centre, Amersham, England. After incubation the ovaries were fixed in ethanol-acetic acid (3 : 1) or Bouin, dehydrated, cleared and embedded in paraffin wax. 4—6 μm sections were cut on a Spencer microtome and mounted onto glass slides (not acid cleaned) previously subbed with Meyer's Glycerine albumine. Dry preparations were coated at 45°C with Kodak NTB2 liquid emulsion diluted 1 : 1 with water. They were then placed in light-tight boxes and exposed for 7—15 days at 22°C. Autoradiographs were developed in Brussels amidol (one part developer to two parts water) and the emulsion fixed in 3 successive changes of 24% sodium thiosulphate, etc. Autoradiographs were stained with Bélanger's stain, dehydrated in ethanol, cleared in xylen and mounted in balsam [1].

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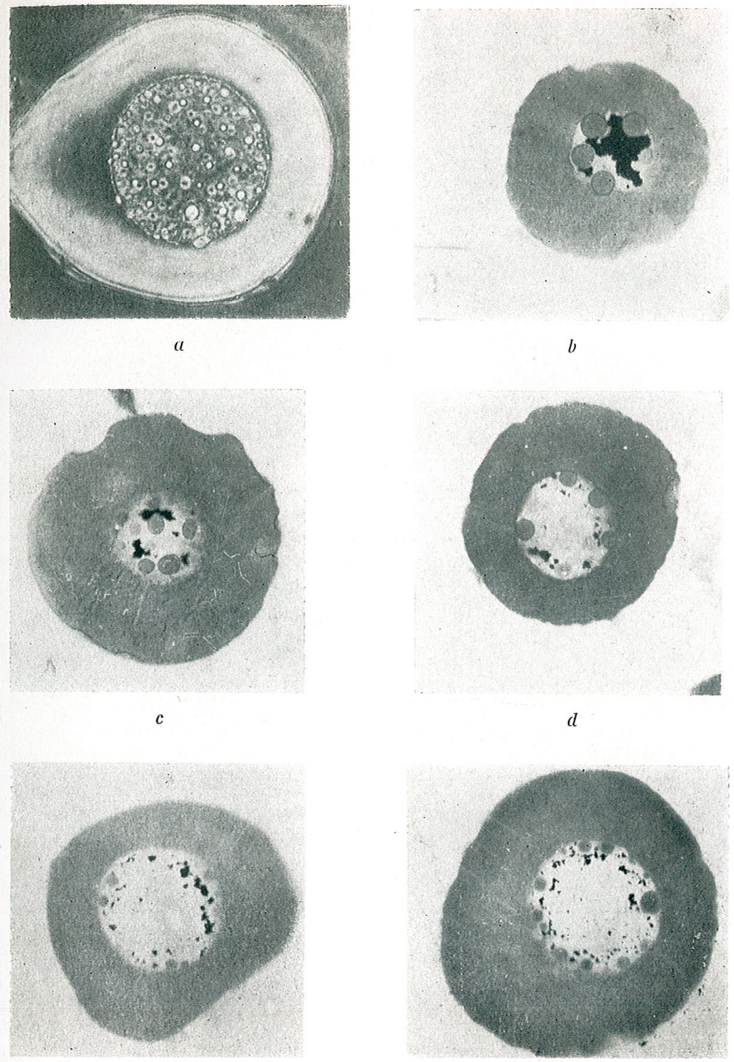
## RESULTS AND DISCUSSION

Small ∅ 0.02 mm oocytes are seldom found, they occurring on ovigerous slides. They have a compact nucleus which strongly incorporates $^3$H thymidine on the inner surface of the nuclear envelope. As the oocytes grow reaching 0.15 mm in diameter, the number of big nucleoli increases in the nucleus showing a rich perinucleolar labeling toward the centre of the nucleus. We have never detected intranucleolar tracers in some amphibians, for instance [8]. On serial sections, tritiated thymidine labeled areas were not found to stretch throughout the free surface of the nucleoli, i.e. the whole surface of the nucleoli, except for the zone directed to or lying on the inner nuclear envelope. During small growth the tracer covers, on certain levels, a large and thick surface on one part of the inner side of the nuclear envelope. That surface comprises neoformative nucleoli. There is no question of attributing such images to lampbrush chromosomes, because the latter lie in the middle of the nucleus, more exactly in the central portion of the nuclear matrix and have no such contiguity with the periphery of the nucleus. It is very likely that this replicating DNA, which actually is a rDNA, should be represented by extracopies of free ribosomal cistrons which continue to replicate in a cascade pattern under the nuclear envelope. Nevertheless, these strongly labeled zones have rather a dispersed DNA to release a Feulgen positive reaction and, as a proof that they are not an artifact of fixation is their being distributed on a section in all directions. This eliminates the objection that labeled DNA could have been pushed by the microtome knife in the respective direction. The count of nucleoli *in situ* in oocytes growing from 0.02 to 0.15 mm/∅ indicates a number of 2—3 to 980—1290 when arylesterase-mucopolysaccharide vacuoles with "acid phospatases" occur and when rDNA replication ceases. At the end of the small growth of oocytes and at the beginning of vitellogenesis, the tracer is reduced to a few points around the perinucleated nucleoli.

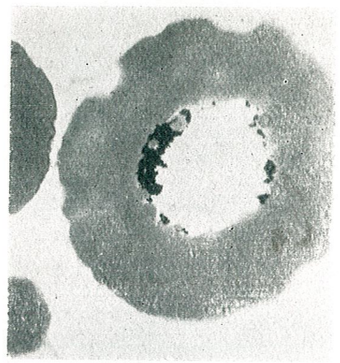
## DISCUSSION

It has been well established that the premeioses "S" phase of DNA synthesis ensuring a quantity of 4 c chromosomal DNA, occurs prior to the two meiotic divisions. Thus, the respective "S" phase in *Triturus vulgaris* takes place 1—2 days after the last spermatogonic mitosis up to the leptotene stage. Premeiotic "S" phase was estimated in the ovaries of *Xenopus Laevis* tadpoles to last from 1 to 2 weeks just before metamorphosis. A series of works demonstrate through autoradiography, molecular hybridization and gradient DNA isolation that after this phase of chromosomal DNA synthesis, there occurs, during oogenesis, a quantity of DNA which grows reaching 20—30 mmg in the pachytene stage of young amphibians [3, 6]. Despite the fact that the DNA isolated from the ovarian tissues contains, in addition to oocyte material, DNA from follicle and stromal cells, the ovarian DNA still contains more than ten times as many ribosomal cistrons as red blood cells DNA. More precisely, in complete ovaries with follicle cells, one finds 20—30 times more rDNA



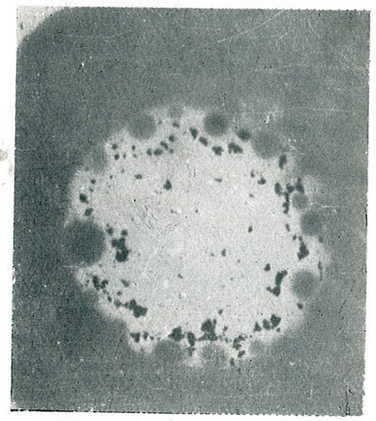
*a* *b* *c* *d* *e* *f*

Plate I. — *a.* oocyte *in vivo* in the diplotene stage, phase contrast (× 1000); *b.* oocyte at the beginning of small growth, with abundent labeled granules and a few nucleoli; *c.* multiplication of nucleoli and decrease of perinuclear tracer; *d, e, f.* dispersion of perinuclear nucleoli with the respective tracers;

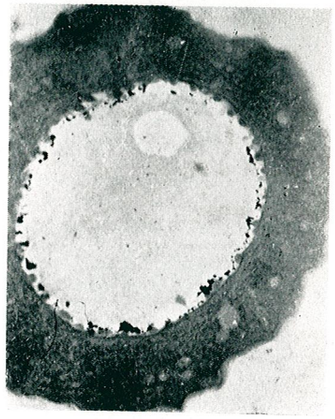


*g*

*h*



*i*



*j*

Plate I. — *g, h, i.* rDNA labeled areas on the inner surface of the nuclear envelope, comprising neoformative nucleoli ; *j.* conclusion of small growth with reduction of perinucleolar tracer.



than in the germinative vesicles which have 10,000—20,000 times the quantity of chromosomal 4C [6]. In any case, the biosynthesis of this DNA takes place in amphibian oocytes in the form of an amplification of cistrons 28 and 18S of ribosomal RNA. Amplification starts in the oogonium and proceeds slowly up to the pachytene stage. Rapid synthesis of DNA takes place in the pachytene and the early diplotene stages over a period of about 20 days. It seems likely that DNA synthesis proceeds in a cascade pattern such that each new replica itself serves as a template for further replication. Although it has been shown that RNA-dependent DNA polymerase might be involved in amplification of the genes (r-DNA) that specify the sequence of ribosomal RNA during the early oogenesis of *Xenopus* [4], this enzyme is specifically inhibited by 2′, 5′-dimethyl-N (4′)benzyl-N (4′)-/dismethyl/rifampicine [5]. These however, are proofs that in other cases, too, the quantity of nucleolar DNA increases in the nucleoli, e.g. in the somatic tissues. Autoradiographic analysis [9] performed by Vlad in very young ovary squashes of Salmo videus has shown that nucleolar DNA synthesis begins in the oogonium and goes on slowly in the leptotene and zigotene stages so that in the pachytene $^3$H thymidine is rapidly and very strongly incorporated. In the early diplotene stage it stops completely when nucleolar DNA becomes dispersed over the inner surface of the nuclear envelope in the form of small Feulgen+granules. On the other hand, we have shown that the phenomenon of gene amplification in *Carassus* is very weak in the leptotene, zigotene, and pachytene stages, while in the diplotene one the quantity of tracers is enormous and keeps so throughout small growth. This aspect has not been followed by the other authors, but we detected it on paraffin sections. As the number of nuclei increases and their volume decreases, they are being surrounded by the tracer. We wish to say that the Feulgen reaction is not sensitive enough to reveal the dispersion of replicative DNA. In fishes, as a rule, at the beginning of small growth a series of granules and Feulgen + perinuclear fringes can be evidenced in the diplotene stage, but they do not match the tracer either in surface or volume (during small growth, large surfaces of labeled DNA comprising nucleoli are visible ; however, because of DNA dispersion, they do not yield a Feulgen positive reaction). As the oocytes begin to grow bigger, that is, as esterase-polysaccharide vacuoles are going to appear, the labeled granules become scantier, but always around the nucleoli under the nuclear envelope.

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# OVARIAN INFLUENCES UPON THE THYMUS

BY

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Ovariectomy performed in female Wistar rats, weighing 100±10g, induced an increase by 35% of $^{32}P$ incorporation in the thymus. After the administration of a total dose of 3 mg/100 g b.w. of oestrogens or progestative hormones, $^{32}P$ incorporation in the thymus is significantly lowered, being associated with a decrease of the gland weight.

The relations between the thymus and the endocrine system represent one of the most complex moments of the physiology of the thymus, which plays a central role in cellular mediated immunity (CMI). It is already known that under the influence of sexual hormones, beginning at the moment of puberty, the thymus is subjected to a slow and irreversible involution.

According to Burnet, the age involution of the thymus leads to a depression of CMI mechanisms, including antitumoral immunity [1].

We investigated, in this connection, the effect of oestrogen and progestative hormones on thymus reactions, which might affect its immunologic capacity.

## MATERIAL AND METHODS

White female Wistar rats weighing 100 ± 10 g were divided into the following groups:

1. control;
2. ovariectomized and taken into experimentation at two weeks from the operation;
3. intramuscularly treated group with a total dose of 3 mg/100 g b.w. of ovocydine $^{R}$ — CIBA (follicular hormone for three days;
4. similarly treated as animals in group 3, but with lutocycline $^{R}$ — CIBA (a progestative synthetic hormon).

Twenty-four hours befor being killed by chloroform, the animals were subcutaneously injected with 5 μCi/100 g, $H_2Na^{32}PO_4$. The thymus was weighed on a torsion balance, digested with alkali and placed, in small portions (0.2 ml) on special plates. Their radioactivity was determined with the aid of a B-2 installation using a CTC-6 gauge at 1200 V.

## RESULTS AND DISCUSSIONS

Table 1 shows that bilateral ovariectomy induces after 14 days a thymic hyperplasia, as the incorporation of $^{32}P$ increases by 35% ($p < 0.001$). According to Milcu and Potop [4] the $^{32}P$ test for the thymus

function may be compared with the $^{131}I$ test for the thyroid, since the thymus the nucleic acid metabolism is very intense. At the same me, the thymus weight increases only by 14.4% ($p < 0.05$). Comşa [ reported an increase by 40% of the thymic hormone in castrated guin pigs.

*Table 1*

**Changes of rat thymus weight and of $^{32}P$ incorporation into the thymus o controls (C), ovariectomized (−0), treated wtih ovocyclin (+0), and litocyclin (+L), animal**

| GROUP | | Thymus weight (mg) | dpm/100 mg tissue (wet weight) |
|---|---|---|---|
| C | $\bar{X}\pm SE(n)$ | 194±6(11) | 786±44(11) |
| −0 | $\bar{X}\pm SE(n)$ | 222±14.2(12) | 1062±51.8(12) |
| | D% | +14.4 | +35 |
| | p | <0.05 | <0.001 |
| +0 | $\bar{X}\pm SE(n)$ | 86±3.5(11) | 530±19.1(11) |
| | D% | −55.7 | −32 |
| | p | <0.001 | <0.001 |
| +L | $\bar{X}\pm SE(n)$ | 121±4.2(11) | 645±36.8(11) |
| | D% | −37.8 | −19.5 |
| | p | <0.001 | <0.05 |

The antagonism between the female gonad and the thymus clear appears also from the experiments with hormone administration. Thu both tests: weight changes and incorporation of $^{32}P$, show a thym involution which is faster after follicular hormone than after progestativ hormone administration. According to Comşa [2] and Pora and Tom [5], the oestrogens might act directly upon the thymus; such an action also suggested by *in vitro* and *in vivo* experiments showing a pharmacol gical lysis. Milcu suggested also that the involutive effect of oestroge on the thymus might affect other components of the endocrine syste like the hypophysis, the thyroid and the adrenals [3].

To conclude, one may say that the thymus-gonadal antagonism ca easily be seen after ovariectomy or administration of oestrogens or pro gestative hormones, by means of both weight and $^{32}P$ incorporation test The involution induced by sexual hormones might also change the immu nologic potential of the thymus, particularly during advanced age.

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# DIE HETEROGENITÄT DER FREIEN HÄMOGLOBINE VON *PROPSILOCERUS DANUBIALIS (DIPTERA, CHIRONOMIDAE)*

VON

ANGHELUŢĂ VĂDINEANU, DUMITRU IGA, GHEORGHE IGNAT

In contrast with other species of *Chironomids* (9—12 components) a lower heterogeneity (4 components) of free hemoglobins from the hemolymph of *Propsilocerus danubialis* larvae were revealed.
A monomeric and polymorphic hemoglobin (Hb 2, a and b components) and a dimeric and polymorphic one (HB 1, aa and bb components) with an estimated MW of 16000 and respectively 32000 were isolated by gel filtration (Sephadex G-75) and polyacrylamide electrophoresis before and after treatment with mercaptoethanol and dodecylsulfate.
The marked tendency to form dimeric structure can explain the incapacity of these larvae to take up oxygen when its partial pressure is lower than 10 mm Hg.

Die Larven von *Propsilocerus danubialis* [10], [1] bevölkern die Ökosysteme der Gewässer des Donau-Seedeltas, wobei sie einen Hauptbestandteil der Benthos-Lebensgemeinschaften in diesen Ökosystemen darstellen. Um erklären zu können, wie Populationen dieser Art an der Grenzfläche Sediment-Wasser auf die Fluktuationen der Sauerstoffkonzentration reagieren, haben wir uns vorgenommen, die Hämoglobine aus der Blutlymphe der Larven zu studieren.

Von den Atmungspigmenten der Wirbellosen, die eine hervorragende Rolle als Sauerstoffspeicher spielen, sind am vollständigsten die freien Hämoglobine aus der Blutlymphe von Larven der Gattung *Chironomus* studiert worden. So kennt man 10—11 mono- und dimere Komponenten aus der Blutlymphe der Larven von *Chironomus thumi* [3], [2]; 9 mono- und dimere Komponenten aus der Blutlymphe der Larven von *Chironomus plumosus* [6], [8]; und 9 ausschließlich monomere Komponenten in der Blutlymphe der Larven von *Chironomus tentans* [9]. Diese Untersuchungen haben ergeben, daß Unterschiede der Molekulargewichte und der Aminosäuren-Zusammensetzung der abgesonderten Hämoglobine bestehen, was auf die Existenz eines Genkomplexes hindeutet, welcher den Aufbau der Polypeptidketten steuert.

## MATERIAL UND METHODE

Die *P. danubialis*-Larven, des Stadiums IV, wurden aus den Delta-Seen Roşu und Puiu eingesammelt, mit destilliertem Wasser gespült und in einem 0,05 M Phosphatmedium, pH 7, homogenisiert.

Das Präparat wurde 30 Minuten lang bei 20 000 U/min zentrifugiert, worauf der Überstand mit der Hämolymphe und den freien Hämoglobinen abgegossen und durch eine Sephadex G 75-Säule von 45 cm Länge und 1,1 cm ø bezw. 90 cm Länge und 1,1 ø filtriert wurde. Die Äquilibrierung und Elution der Säulen wurde mit 0,05 M Phosphatpuffer bei pH 7 durchgeführt; die Filtrationsgeschwindigkeit war auf 20 ml/h eingestellt. Von den erhaltenen Fraktionen (1,5 ml) wurden mit einem Specord-Gerät die Spektren aufgezeichnet.

Mit Hilfe der Spektren wurden die Verhältnisse $E_{415}/E_{280}$ berechnet, worauf die Fraktionen mit deutlich ausgebildeten Maxima bei 415 nm (Oxyhämoglobin) elektrophoretisch analysiert wurden.

Die Elektrophorese der Hämoglobine enthaltenden Fraktionen geschah in Polyacrylamid-Gel [5]. Die Dissoziation der dimeren Hämoglobine wurde durch Behandlung mit Mercaptoethanol und Dodecylsulfat 1%-ig erreicht [11]. Die Anwensenheit der Hämoglobine in den Polyacrylamid-Gelen wurde mit Hilfe der Peroxidase-Reaktion festgestellt [4].

Zwecks schätzungsweiser Bestimmung des Molekulargewichtes der Monomeren wurde ein Gemenge aus $Hb_2$ und Lysozym (MW = 13 000) in 0,05 M Phosphatpuffer (pH 7) durch eine Sepadex G 75-Säule filtriert.

## ERGEBNISSE UND DISKUSSION

In dem Filtrat des Überstandes durch eine Sephadex G 75-Säule (L = 45 cm, 1,1 cm ø) konnten durch Absorbtion bei 280 nm zwei Peaks und bei 415 nm ein einziger Peak ausgemacht werden (Abb. 1). Die geringen Abmessungen der Säule haben die Trennung der Hämoglobinkomponenten in dem Peak II entsprechenden Filtrat nicht gestattet. Durch Elektrophorese dieses Filtrats in Polyacrylamid-Gel konnte mittels der Peroxidase-Reaktion die Existenz von vier Komponenten — davon zwei Hauptkomponenten — festgestellt werden (Abb. 2).

Durch Verwendung einer längeren Säule (L = 90 cm, 1,1 cm ø) wurde versucht, die Hämoglobinkomponenten verschiedenen Molekulargewichts voneinander zu trennen.

Wie aus Abb. 3 zu ersehen ist, löste sich der zweite Peak bei 415 nm in zwei Peaks auf, was die Existenz zweier Hämoglobintypen mit verschiedenen Molekulargewichten anzeigt.

Das Bestehen der beiden Hämoglobintypen nach dem Molekulargewicht wird auch durch das Verhältnis $E_{415}/E_{280}$ gestützt, dessen Wert für $Hb_1$ unter eins liegt und für $Hb_2$ gleich eins ist. Nachdem aus Abb. 3 hervorgeht, daß $Hb_1$ über 90% des Gesamthämoglobins darstellt, haben wir die Annahme gemacht, daß es den beiden Hauptbändern (Abb. 2) entspricht, und haben diese Hypothese durch die elektrophoretische Trennung von $Hb_1$ unter denselben Bedingungen geprüft. Das Elektrophorese-Schaubild (Abb. 4) stellt den Polymorphismus des Hämoglobins 1 ($Hb_1$) heraus, der durch zwei Hauptkomponenten bestritten wird.

Aus dem Obigen ergibt sich klar, daß auch $Hb_2$ polymorph ist und in Abb. 2 durch die zweitrangigen Komponenten vertreten ist.



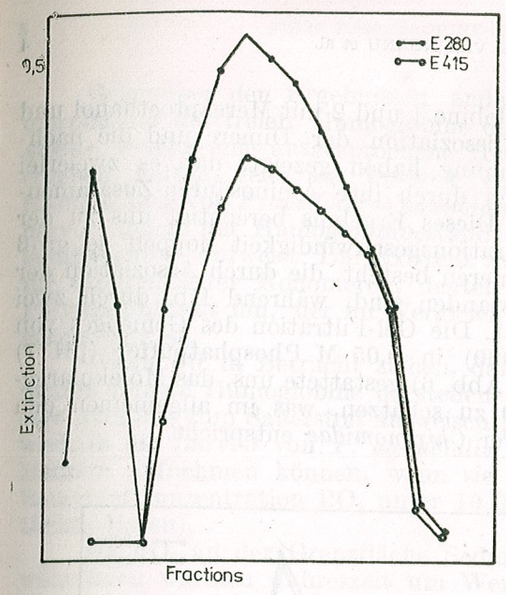


Abb. 1 — Die Trennung des Supernatanten an einer Sephadex G 75-Säule (L = 45 cm, Ø = 1,1 cm).

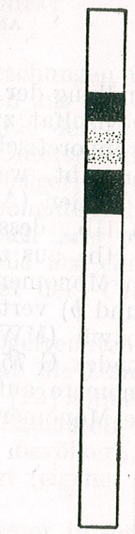

Abb. 2 — Polyacrylamid Gel Elektrophorese des Peaks bei 415 nm, entsprechend Abb. 1.

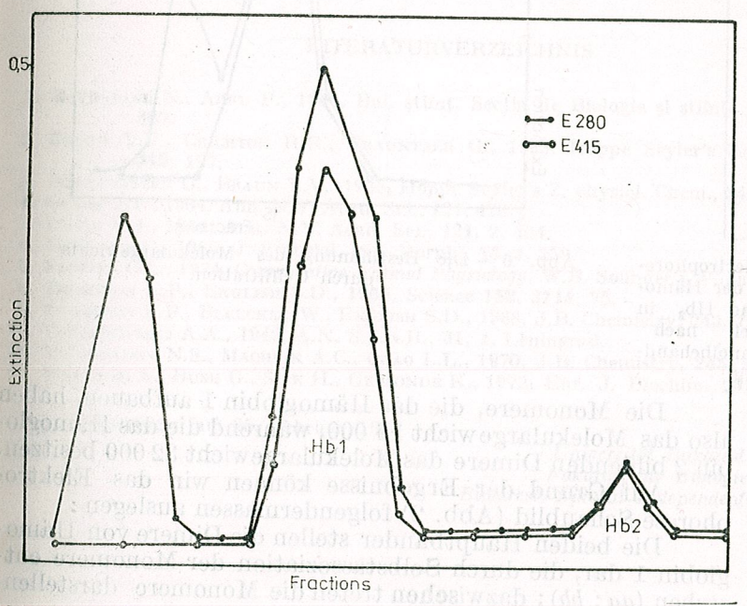


Abb. 3 — Die Trennung des Supernatanten an eine größeren Sephadex G 75-Säule (L = 90 cm, Ø = 1,1 cm).

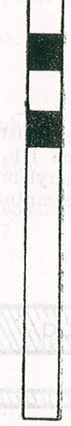

Abb. 4 — Elektrophoretische Trennung der Hämoglobine entsprechend dem ersten Peak Abb. 3.

Die Behandlung der Hämoglobine 1 und 2 mit Mercaptoethanol und 1%-igem Dodecylsulfat zwecks Dissoziation der Dimere und die nachfolgende elektrophoretische Trennung haben gezeigt, daß es zweierlei Monomer-Typen gibt, welche sich durch ihre Aminosäuren-Zusammensetzung unterscheiden (Abb. 5). Dieses Ergebnis berechtigt uns zu der Annahme, daß $Hb_1$, dessen Filtrationsgeschwindigkeit doppelt so groß ist als die von $Hb_2$, aus zwei Dimeren besteht, die durch Assoziation der nachgewiesenen Monomeren entstanden sind, während $Hb_2$ durch zwei Monomere (*a* und *b*) vertreten ist. Die Gel-Filtration des Gemenges von $Hb_2$ mit Lysozym (MW = 13 000) in 0,05 M Phosphatpuffer (pH 7) durch die Sephadex G 75-Säule (Abb. 6) gestattete uns, das Molekulargewicht der Monomere auf 16 000 zu schätzen, was im allgemeinen den Werten für die Monomere anderer *Chironomidae* entspricht.

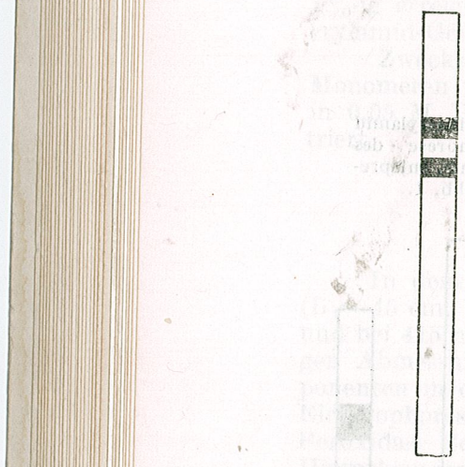

Abb. 5 — Electrophoretisches Muster der Hämoglobine $Hb_1$ und $Hb_2$ in Polyacrylamidgel nach Mercaptoäthanolbehandlung.

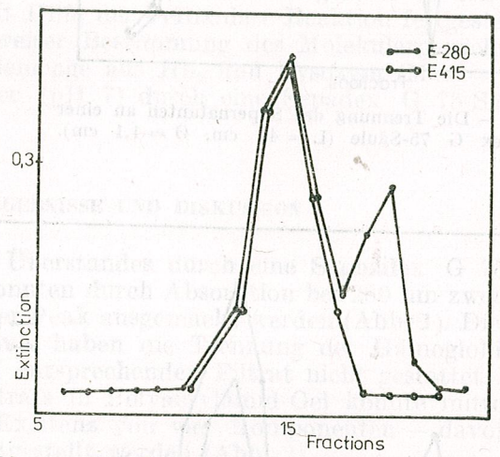


Abb. 6 — Die Bestimmung des Molekulargewichts durch Gelfiltration.

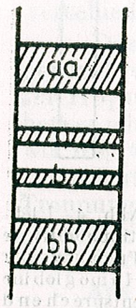


Fig. 1A

Die Monomere, die das Hämoglobin 1 aufbauen, haben also das Molekulargewicht 16 000, während die das Hämoglobin 2 bildenden Dimere das Molekulargewicht 32 000 besitzen.

Auf Grund der Ergebnisse können wir das Elektrophorese-Schaubild (Abb. 2) folgendermassen auslegen:

Die beiden Hauptbänder stellen die Dimere von Hämoglobin 1 dar, die durch Selbstassoziation der Monomere entstehen (*aa*; *bb*); dazwischen treten die Monomere darstellenden Bänder *a* und *b* auf.

Die Monomere des Hämoglobins von *P. danubialis* bekunden eine ausgesprochene Assoziationstendenz, weshalb das Hämoglobin dieser Larven hauptsächlich aus Dimeren besteht.



Gegenüber den Ergebnissen anderer Forschungen über den Polymorphismus der freien Hämoglobine der Larven aus der Gattung *Chironomus*, welche eine ausgesprochene Heterogenität der Hämoglobintypen ergeben hatten, geht aus unseren eigenen Untersuchungen über die Hämoglobine von *Propsilocerus danubialis* eine viel geringere Heterogenität hervor, indem der Hämoglobinbestand 4 Komponenten, davon 2 Hauptkomponenten, aufweist. Die Existenz zweier, nach ihrer primären Struktur differenzierter Monomeren, deutet gleichfalls auf einen genetischen Polymorphismus hin, der im Vergleich zu dem der *Chironomus*-Arten geringfügig ist.

Wenn wir in Betracht ziehen, daß die abgetrennten Hauptkomponenten dimere Hämoglobine darstellen, und, daß die Dimere eine geringere Affinität zum Sauerstoff aufweisen [7], [6], [12] können wir erklären, weshalb die Larven von *P. danubialis* keinen Sauerstoff mehr aus dem Medium aufnehmen können, wenn sie Medien bevölkern, in denen die Sauerstoffkonzentration $PO_2$ unter 10 Torr liegt (eigene, nicht veröffentlichte Daten).

Da $PO_2$ an der Grenzfläche Sediment-Wasser in der 4—5 Monate währenden warmen Jahreszeit um Werte schwankt, die 13 Torr nicht überschreiten, sind die Hämoglobine dieser Larven unwirksam und diese leben unter praktisch anaeroben Bedingungen, was den Stillstand ihres Wachstums in diesem Zeitraum erklärt.

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# EFFECT OF WHOLE BODY TRRADIATION WITH NEUTRONS ON HAEMOGLOBIN

BY

MIOARA CÂRSTEANU, C. VLĂDESCU, E. IANCU

**In this paper the authors have studied by electrophoretic method the influence of whole body irradiation of rats, with different neutron doses, on blood haemoglobin. The irradiation of animals leads to modifications of haemoglobin at molecular level that are in relation with the dose.**

The development of biological tissue irradiation with neutrons, for diagnostic or therapeutic purpose, requires a detailed investigation of radiobiological effects.

The first report about the radiobiological effects of neutrons was mentioned by Lawrance and collab. [6]. The authors pointed out that whole body irradiation of rats with X-rays and neutrons destroys the haematopoetic and lymphoid tissues, of the small intestine, etc.

Later investigations in neutron radiobiology contributed to the possibility of their utilization in medicine and biology.

Available data indicate that after a neutron exposure cells scarcely recover and the chemical protectors have only a slight effect on the restitution process [7].

For this purpose it is necessary to determine some biological thresholds in irradiated organism before the appearance of effects that can be detected with usual methods; so, in our laboratory we studied the effect of whole body irradiation with neutrons in different doses, on haemoglobin because of the physiological and biochemical importance of this macromolecule.

## MATERIAL AND METHODS

Animals: Wistar rats, 10—12 months old and 150 gr weight. The animals were irradiated with 600, 300, 200 and 150 r fast neutrons, and sacrificed after one, two, three, four, five and thirty days. Neutron source: beryllium target bombarded with deuterons accelerated in the cyclotron for an energy of 13.5 MeV. The neutron flow, corresponding to a 1 $\mu$A accelerated deuterons current, was $\Phi = 1.63/10^8$ n/cm$^2$ sec. The dosimetric equivalent of the neutron flow, 1 rad/sec., corresponds to a flow of $2 \cdot 10^8$ n/cm$^2$.sec. The dose flow was 0.81 rad/sec.

In condition in which the deuteron-current intensity is the same, the variation of the dose will be given by the variation of the exposure

time (in our case 6 min for a dose of 600 rad, 3 min for a dose of rad, 2 min for a dose of 200 rad and 1.5 min for a dose of 150 neutrons).

The disk-electrophoresis method Clark [2] was employed for neutron irradiation effect on haemoglobin, using a 6% polyacrylamide Solution A : 30 g acrylamide monomer, 1 g N, N′-methylenbis-acrylan and 123 ml distilled water. Solution B : 0.28% N-N-N′-N′-tetramethy diamine. Solution C : 0.14 g ammonium persulphate. Solution D : 2 glycine, 6 g tris and 980 ml distilled water. The proportion of gel mixtu 2 vol. solution A + 1 vol. solution B + 4 vol. solution C + 1 vol. solut D. The buffer is formed by mixing 29 g glycine, 6 g tris, 5 ml HC and 975 ml distilled water, pH 8.1. The buffer is diluted 1 : 10.

To the empty well at the top of the vertically supported gel t are added 0.3 ml of 5% sucrose solution that contains 0.02 ml eryth cytes haemolysate. Electrophoresis is carried out, by applying a curr of 1.2 mA per tube for 1—2 hours. When the electrophoresis is complet the gels are immediatelly removed from the glass tubes and stained w a benzidine reagent : 0.2 ml of 30 per cent hydrogen peroxide is ad to a solution of 0.2 g benzidine and 0.5 ml acetic acid in 100 ml wa just before use. Characteristic blue bands form within 30 minutes usually fade to dark-brown upon subsequent storage in water. Haem globin preparation : the rat blood is added on salt-EDTA (Na-diamino thantetraacetic acid) and is centrifugated for 10 min at 1500 r.p.m. T sediment is washed with 0.15 M Na-chloride and then is centrifuga with 1.5 vol. distilled water at 4°C and 0.5 vol. toluol at 6000 r.p.m. 20 min.

## RESULTS

Haemoglobin from normal rats leads to five electrophoretic fr tions (Fig. 1).

The same electrophoregrams as in controls are obtained 24 ho after an irradiation of rats with 600 rad neutrons (Fig. 1), but the elect phoretic mobility of each band is decreased with approximately 30%. Th means a lower molecular weight of each type of haemoglobin in relat with the mobility decrease of each band.

After 48 hours, all the bands are diffuse ; after 72 hours, the elect phoregram shows only the first band, the other four bands forming diffuse mass (Fig. 1).

24 hours after the irradiation of animals with 300 rad neutrons, th appear five bands on the eletrophoregrams like in controls (Fig. 2). Af 48 hours, only the first band is clearly separated ; bands 4 and 5 are diffu bands 2 and 3 form a diffuse mass. When the animals are sacrificed 72 ho after irradiation with 300 rad neutrons, the electrophoregram reveals w difficulty only bands 1, 4 and 5. After 96 hours, the electrophoretic mo lity of the first band is depressed with approximately 19%, like in contr the others being very diffuse.

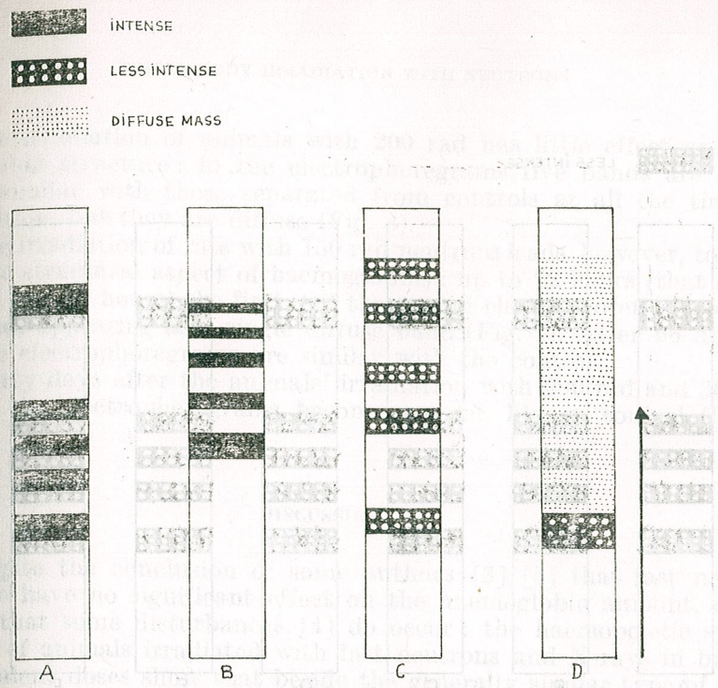


Fig. 1. — Irradiation with 600 rad neutrons ; *A* — controls ; *B* — after 24 hours ; *C* — after 48 hours ; *D* — after 72 hours.

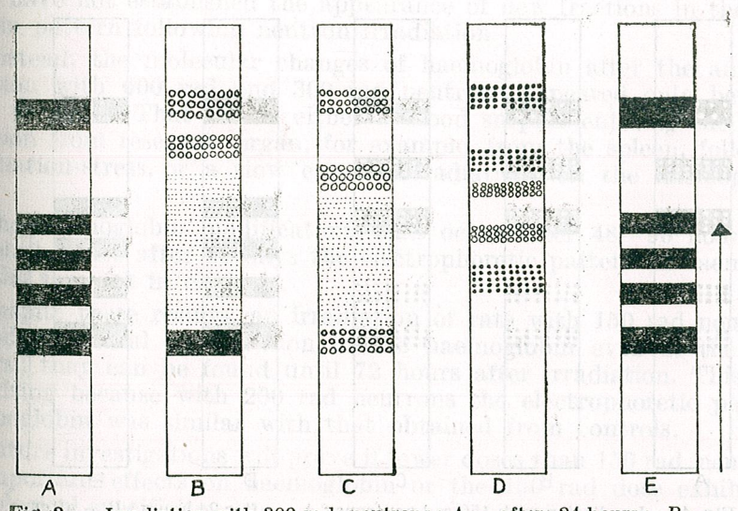


Fig. 2. — Irradiation with 300 rad neutrons ; *A* — after 24 hours ; *B* — after 48 hours ; *C* — after 72 hours ; *D* — after 96 hours ; *E* — after 30 days.

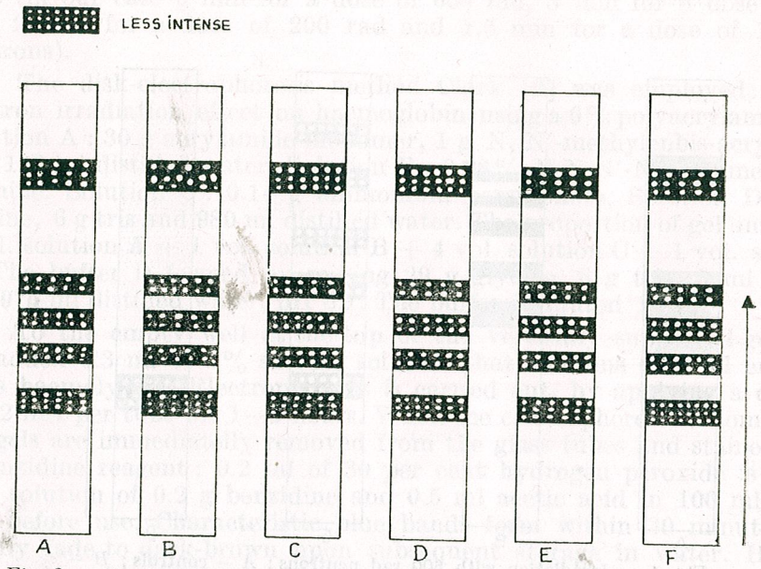


Fig. 3. — Irradiation with 200 rad neutrons ; *A* — after 24 hours ; *B* — after 48 hours ; *C* — after 72 hours ; *D* — after 96 hours ; *E* — after 144 hours ; *F* — after 30 days.

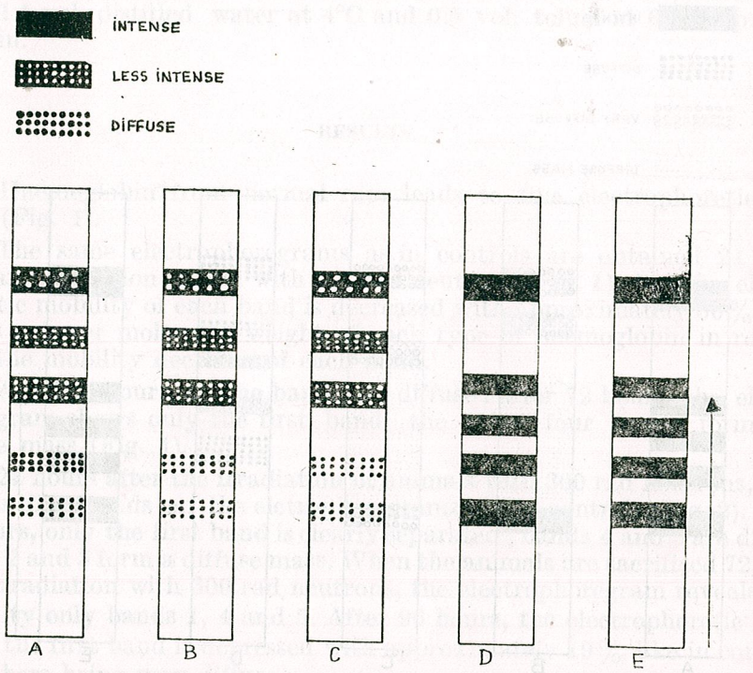


Fig. 4.— Irradiation with 150 rad neutrons ; *A* — after 24 hours ; *B* — after 48 hours ; *C* — after 72 hours ; *D* — after 96 hours ; *E* — after 144 hours.



The irradiation of animals with 200 rad has little effect upon the aemoglobin structure : in the electrophoregrams five bands are distinuished similar with those separated from controls at all the times of nvestigation, but they are diffuse (Fig. 3).

The irradiation of rats with 150 rad neutrons leads, however, to chanes in the structural aspect of haemoglobins : up to 72 hours (that means t 24, 48 and 72 hours) the first and the second electrophoretic bands are ot bound, appearing as a single diffuse band (Fig. 4). After 96 and 144 ours the electrophoregrams are similar with the control.

Thirty days after the animals' irradiation with 200 rad and 300 rad eutrons the electrophoregrams become normal, like in control (Figs 2 nd 3).

## DISCUSSION

Despite the conclusion of some authors [3] [5] that fast neutron adiations have no significant effect on the haemoglobin amount, others onsider that some disturbances [4] do occur : the haemopoietic system eactions of animals irradiated with fast neutrons and X-rays in biologial equivalent doses show that beside the generally similar type of chanes, certain differences also exist. Compared to X-irradiation, erythrocyte esistance in fast neutron irradiated animals was markedly lower, with ome changes in the structural pattern of the haemoglobin molecule, too. hebotarev and collab. [1], Sobels and collab. [8] have mentioned some odifications of haemoglobin concentration in the blood following neuon irradiation.

Starodub [9] has studied the heterogenity of rat haemoglobin by isc-electrophoresis method before and after neutron irradiation in lethal ose. Our results are partially similar with those obtained by Starodub, ut we have not established the appearance of new fractions in the haeoglobin pattern following neutron irradiation.

Instead, the molecular changes of haemoglobin after the animals' radiation with 600 rad and 300 rad neutrons appeared only between 4 and 48 hours. That means either a blood surplus entering the blood rculation from reservoir organ, for example from the spleen, following ie radiation-stress, or a slow effect of radiations on the haemoglobin self.

The haemoglobin modifications that occur after 48—96 hours are versible because after 30 days the electrophoretic pattern of haemoglon is the same as in controls.

Despite these results, an irradiation of rats with 150 rad neutrons oduced structural modification in the haemoglobin even in the first hours ; they can be found until 72 hours after irradiation. This fact surprising because with 200 rad neutrons the electrophoretic pattern haemoglobin was similar with that obtained from controls.

Future investigations will prove if lower doses than 150 rad neutrons ve important effects on haemoglobin or the 150 rad dose exhibits a aiobiological specificity.

In conclusion, whole body irradiation with doses between 600—
rad neutrons has an important effect on the molecular structure of hae
globin; this effect is in relation with the dose.

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# HE EFFECT OF ALLOXAN, STREPTOZOTOCIN AND OBALT CHLORIDE UPON THE GLUCOSE CONTENT IN THE HAEMOLYMPH OF *MYTILUS GALLOPROVINCIALIS* (L.)

BY

IOSIF MADAR, NINA ŞILDAN, EUGEN A. PORA

It has been established that 24 hours after administration of beta-cytotoxic agents (alloxan 12.1 mg or streptozotocin 20 mg/100 g b.w.) the level of haemo-lymphatic glucose in sea mussels is significantly increased. Cobalt chloride, an alfa-cytotoxic, 6 hours after its administration (20 mg/100 g b.w.) also increased the glucose content in the haemolymph. The possible role of insulin- and glucagon-like substances in the carbohydrate metabolism of sea mussels is discussed.

Recent data indicate that in the gastrointestinal mucosa of some alve molluscs insulin-like producing cells are present [2], [3], [11], 2], [14]. Furthermore, from our preliminary observations results that intestinal mucosa of *Mytilus galloprovincialis* contains islet formations which, by the classical Gömöri technique, insulin-like producing baso-yl cells and glucagon-like secreting acidophyl cells can be detected [11]. ese findings led us to study the effect of alloxan and streptozotocin th well known as beta-cytotoxics) as well as the effect of cobalt chlo-e (an alfa-cytotoxic agent), upon the glucose content in the haemo-aph of sea mussels.

## MATERIAL AND METHODS

The experiments were performed on a total number of 120 mussels h a mean body weight of 14.19 g and mean length of 5.39 cm, corres-ding to the age of 2.5 to 3.0 years [8]. The animals were collected m the low water of the Romanian sea-shore and kept for 3—5 days sand-filtered and aerated sea water prior to the experiments, the tem-ature of water being 22°C, and total salinity of 1.5%, respectively.

All experiments were carried out between 14 and 26 June 1979 the laboratories of the Romanian Institute for Marine Research, Con-nța-Agigea. The animals were divided into the following groups:

*Group I*: Controls, injected with saline solution;

*Group II*: Mussels injected with a single dose of 12.1 mg alloxan 100 g b.w.;

*Group III*: Mussels injected with 20 mg streptozotocin per 100 g .;

*Group IV* : Mussels injected with 20 mg cobalt chloride per 100 g b

Alloxan (p.a. "Austranal") and $CoCl_2 \cdot 6\ H_2O$ (p.a. "Merck") w dissolved in saline solution (filtered and sterilized natural sea wat while streptozotocin ("Boehringer" Mannheim, GmbH) was dissolved 50 mM sodium citrate containing saline solution (pH = 4.5).

The compounds were always freshly dissolved and injected i the hepatopancreas *via ligament*, giving a total volume of 50 µl solut per 10 g b.w for each individual, using a microsyringe with needle No.

Samples of 200 µl haemolymph were collected for glucose as 24 hours after injecting alloxan, streptozotocin or saline alone, and 6 ho after administration of cobalt chloride, respectively. For obtaining h molymph, the shells of mussels were opened by unilateral section of *posterior adductor* muscle. The interpallial water was removed by fil paper and the haemolymph was collected in centrifuge tubes, contain 0.7 ml double distilled water. The samples were deproteinized with N Ba $(OH)_2$ and 5% $ZnSO_4$, according to the method of M. Somog modified by N. Nelson [13]. After centrifugation for 10 minutes at 2, rpm of the samples, their glucose content was determined by the GO Perid method of W. Werner *et al.* [16], using Test-Combination Glucose ("Boehringer" Mannheim, GmbH).

The optical density of samples was measured at 610 nm, us a spectrophotometer "Spekol" (Carl Zeiss, Jena). The data expressed mg glucose/100 ml haemolymph were calculated and mean values w compared to the controls. The differences were considered statistica significant at $P < 0.05$.

## RESULTS AND DISCUSSIONS

The data summarized in Table 1 indicate that the concentration glucose in the haemolymph of control mussels is 12.01 ± 0.38 mg/100 This value is almost similar to that obtained by us under basal experim tal conditions [9] and underlines our conclusion that inside an age-gr of sea mussels the glycemia varies only between age-related "homeostat limits [8].

*Table 1*

**Mean values±S.E. of glucose content in the haemolymph of *Mytilus galloprovincialis* (L.) under basal condition (Control), 24 hours after administration of alloxan or streptozotocin, and 6 hours following injection of cobalt chloride**

| mg glucose/100 ml haemolymph | | | |
|---|---|---|---|
| CONTROL | ALLOXAN | STREPTOZ. | COBALT |
| 12.01±0.38 (33) | 20.37±1.42 (31) | 26.65±2.38 (24) | 29.32±2.10 (31) |
| Dif. %: _"_ | +69.71 | +121.90 | +144.13 |
| P : _"_ | <0.001 | <0.001 | <0.001 |

Number of experiments is given in parentheses. Per cent modifications are compared to the control.



As compared to the control values, in alloxan- or streptozotocin-injected groups the haemolymphatic glucose content is obviously increased. It seems very likely that these effects are due to the selective destruction by alloxan or streptozotocin of the insulin-like producing basophyl cells in islet formation of the intestinal mucosa of mussels [11], similarly as in mammals [5], [15], phenomenon which is associated with hyperglycemia in response to the diabetogenic action of these beta-cytotoxic compounds [5], [15], [11]. In fact, recent data indicate the presence of insulin-like producing cells in the gastrointestinal mucosa of some bivalve molluscs [2], [3], [12], [14] and insulin-like producing cell containing islet formations in the intestinal mucosa of *Mytilus galloprovincialis* [11]. On the other hand, it has been stated that insulin in *Anodonta cygnea* and *Unio pictorum* has a possible role in the regulation of carbohydrate metabolism [14], while in *Mytilus galloprovicialis* it induces hypoglycemia [9] and stimulates the glucose up take by isolated mantle pieces [10].

As noticed, following administration of a single dose of cobalt chloride, the glucose level in haemolymph is significantly enhanced in comparison with the controls. Taking into consideration that cobalt chloride, by selective destruction of glucagon producing pancreatic islet cells, induces glucagon release and hyperglycemia in mammals [1], [4], [6], [7], it is pertinent to assume that in mussels this alfa-cytotoxic agent affects the glucagon-like producing acidophil cells in islet formations of the intestinal mucosa. We demonstrated elsewhere [11] that in the intestinal mucosa of *Mytilus galloprovincialis*, besides the basophyl cells there are present also, in a reduced number, acidophyl cells [11]. In view of it, it is very probable that the latter have glucagon-like producing function, and are involved in the regulation of the carbohydrate metabolism in this species. For demonstrating the alfa-cytotoxic effect of cobalt chloride, and the beta-cytotoxic effect of alloxan or streptozotocin in sea mussels, our light- and electronmicroscopic studies are in progress.

*In conclusion*, cobalt chloride (an alfa-cytotoxic agent) and alloxan or streptozotocin (beta-cytotoxic compounds) in *Mytilus galloprovincialis* markedly increase the haemolymphatic glucose content, by affecting the regulation mechanisms involved in carbohydrate metabolism.

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# AGE-DEPENDENT CHANGES OF ADRENAL GLAND REACTION IN RATS UNDER REPEATED EXPOSURE TO COLD

BY

ȘTEFANIA MANCIULEA, VICTORIA M. RUSU, AL. VRĂBIESCU

Young and old white Wistar rats were repeatedly exposed to cold (−4°C) for a period of 30 days. Weight and morphometric modifications, of the glycogen content and ascorbic acid concentration as well as the activity of glucose 6-phosphate dehydrogenase and acid phosphatase in the adrenal gland were studied on the 30th day of experiment. The results suggested a different adrenal reaction to cold in terms of age.

The dynamics of changes characterizing the adrenal response to the action of cold or to a whole series of stressors is relatively well known [3], [8], [10]. Thus, it is unanimously accepted that the exposure to cold results in an increase of adrenaline and noradrenaline release during the first moments of the reaction; later, the corticoadrenal hormones intervene their increased secretion being of great importance for the onset of resistance to the action of stress factors [2], [5]. Much less known are the age dependent peculiarities of the adrenal reaction in such conditions.

In this paper, we have studied some aspects of the adrenal reaction in rats of different age repeatedly exposed to cold.

## MATERIAL AND METHODS

White Wistar rats kept under common laboratory conditions, with food and water *ad libitum* were used. The rats, divided into two parallel experimental series comprising animals aged 2 months and 2 years respectively, were daily exposed to −4°C for one hour, in individual cages for a period of 30 days. During the whole experiment the controls were maintained at the usual laboratory temperature (+20°C). The animals were sacrificed on the 30th day of the experiment and the adrenals were adequately prepared in order to study weight and morphometrical modifications, the glycogen content — determined by the Montgomery method [7] — and ascorbic acid concentration by Klimov's technique [6]. We also demonstrated, by histochemical methods, the activity of glucose-6-phosphate dehydrogenase (= G6PDH), according to Altman, 1968 [1] and of acid phosphatase (= Ac.P) according to Bitensky, 1962 [1].

## RESULTS AND DISCUSSION

It is known that age is a principal factor on which the animals' adaptive capacity to cold depends [4], [11]. The present results demonstrate the different reaction to cold of the adrenal gland in the two studied ages. Thus, the repeated exposure to cold does not cause modifications in the absolute and relative adrenal weight in animals aged 2 months, while in 2-year-old animals there is a sensible increase of both absolute and relative weight, in comparison with the control group (Table 1). Simultaneus-

*Table 1*

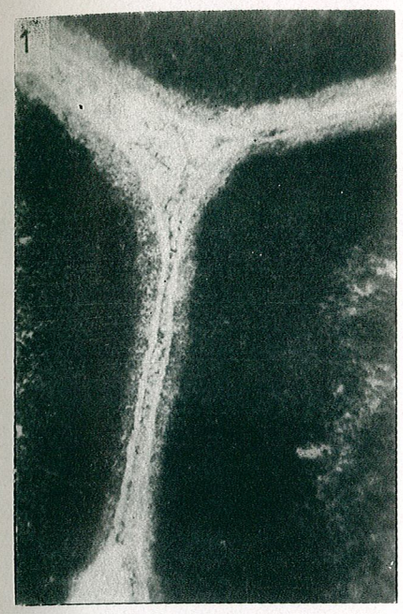
**Variations of the adrenal gland weight and volume in controls and cold-exposed rats**

| | | Rats 2 months old | | Rats 2 years old | |
|---|---|---|---|---|---|
| | | Control | Cold exposed | Control | Cold exposed |
| ABSOLUTE WEIGHT (mg) | M | 57.33 | 56.92 | 58.80 | 80.47 |
| | SE | ±3.20 | ±1.46 | ±3.08 | ±4.66 |
| | n | 12 | 12 | 12 | 15 |
| | P | | >0.05 | | <0.001 |
| RELATIVE WEIGHT (mg/100 g) | M | 26.99 | 27.76 | 23.63 | 34.60 |
| | SE | ±1.48 | ±0.52 | ±1.15 | ±2.40 |
| | n | 12 | 12 | 12 | 15 |
| | P | | >0.05 | | <0.001 |
| DIAMETER (mm) | M | 2.540 | 2.489 | 2.526 | 3.026 |
| | SE | ±0.084 | ±0.087 | ±0.048 | ±0.144 |
| | Pn | | >0.05 | | <0.001 |
| C/M | M | 2.54 | 3.73 | 2.09 | 2.16 |
| | SE | ±0.24 | ±0.36 | ±0.10 | ±1.88 |
| | P | | <0.01 | | >0.05 |

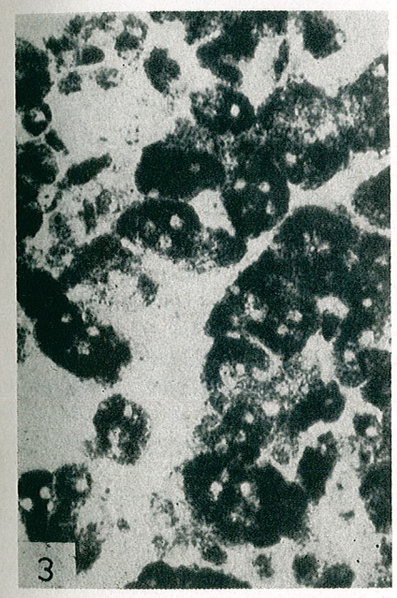
ly with weight changes, variations of the gland volume were observed In young animals exposed to cold one may observe the evident extensio of the cortical surface, also expressed by the increase of the value of th cortical/medulla ratio (= C/M). In 2-year-old animals the value of th C/M ratio is very little changed after exposure to cold, because there is marked increase of both zones of the gland and therefore of its volum (Table 1).

The reaction of the adrenal gland depending on age is also observe in the case of variations of glycogen concentration and of ascorbic aci content (Table 2). The adrenal glycogen concentration in the young ra exposed to cold markedly increases, but it is unchanged in the old one Generally, the repeated exposure to cold determines an increase of th ascorbic acid content in the adrenal gland. In young rats there is onl a tendency to increase, which does not reach the threshold of statistic significance, while in old animals the increase is quite pronounced.

In both groups of animals, the exposure to cold determines the m difications of G6PDH activity in the cortex where the enzyme reach a very high level of the reaction intensity (Fig. 1). But, if in young anima there is an intensification of reaction, in the old animals' cortex th enzyme activity is strongly depressed under the influence of cold (Fig. 2







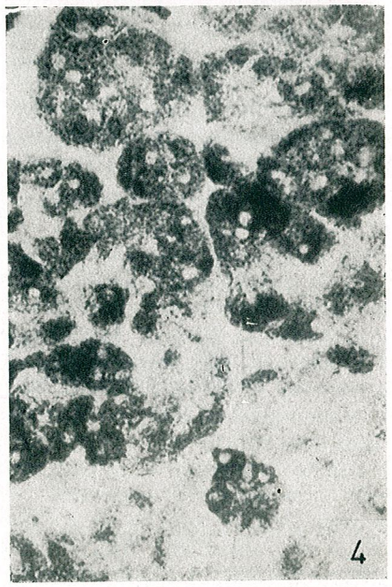




Fig. 1. — Distribution of G6PDH activity in the adrenal gland of young rats.
Fig. 2. — Decrease of G6PDH reaction in the adrenal gland of young rats exposed to cold.
Fig. 3 — The high level of acid phosphatase reaction in the adrenal medulla of old animals.
Fig. 4. — Aspect of acid phosphatase reaction in the adrenal medulla of young animals.

The different response of the adrenal medulla in both age groups is suggested by the modifications occurring in the acid phosphatase activity, which also permits the observation of some differences caused by age in the controls' adrenals. Thus, one may see a numerical increase of intensely phosphatasic cells in the adrenal medulla of old animals, in comparison with young animals (Figs. 3, 4). The modifications caused by cold are also different. In young animals, a slight increase of the acid

*Table 2*

**Variations of glycogen and ascorbic acid concentrations in the adrenals of control and cold-exposed rats**

| | | 2-month-old rats | | 2-year-old rats | |
|---|---|---|---|---|---|
| | | Control | Cold exposed | Control | Cold exposed |
| GLYCOGEN µg/mg | M | 1.41 | 3.60 | 1.01 | 1.12 |
| | SE | ±0.16 | ±0.47 | ±0.19 | ±0.18 |
| | n | 8 | 8 | 7 | 8 |
| | % | | +155 | | +11 |
| | P | | <0.001 | | >0.05 |
| ASCORBIC ACID mg/100 g | M | 344 | 386 | 233 | 350 |
| | SE | ±21 | ±10 | ±16 | ±13 |
| | n | 8 | 8 | 7 | 8 |
| | % | | +12 | | +50 |
| | P | | >0.05 | | <0.001 |

phosphatase reaction is recorded, while in old rats, on the contrary, there is a sensible decrease of the enzyme activity under the influence of cold.

The glycogen accumulation and the exacerbation of G6PDH activity — as an enzyme generating NADP — in the adrenals of young animals exposed to cold, simultaneously with an increased C/M ratio permit the supposition that in these animals cold causes the increase of glucocorticoid biosynthesis, the cathecolamine secretion remaining within normal limits, as certified by the increased activity of acid phosphatase in the gland medulla. On the contrary, in the old animals the increase of the ascorbic acid content and the decrease of G6PDH activity suggest the possible weakening of the corticoadrenal function, and consequently, the decrease of glucocorticoid synthesis. In exchange, the strong decrease of acid phosphatase in the adrenal medulla shows a strong discharge of cathecolamines in this group of animals. Therefore the following hypothesis could be assumed: under the conditions of our experiment, the prevalent part in the processes of resistance and adaptation to cold is played by the adrenal cortex, in young animals, while in the old ones the intervention of cathecolamines and very probably of the thyroid gland remains essential. And this, because according to some published data [5], [8], [9], an increase of the ascorbic acid in the adrenal gland may take place after repeated exposure to cold, as a result of the intensification of the thyroid function together with the weakening of the corticoadrenal function.

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# SPECIES RELATED FUNCTIONAL PROPERTIES OF MITOCHONDRIA: COMPARISON BETWEEN RAT AND HUMAN LIVER MITOCHONDRIA

BY

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A comparative analysis of some functional properties of rat and human liver mitochondria has been performed in order to further the understanding of protein-lipid interactions in mitochondrial membranes of various species. The stimulation of activity of 3-hydroxybutyrate-dehydrogenase (a phospholipid dependent enzyme) by albumin and the specificity of human liver cytochrome oxidase towards the oxidation of human cytochrome *c* might arise from a particular lipid microenvironment of membrane enzymes in human liver mitochondria. It was interesting to find that human liver mitochondria exhibit certain functional properties similar to other lipid-rich mitochondria (from brown adipose tissue), such as the optimal condition for oxidative phosphorylation or the stimulation of adenine nucleotide translocation by albumin and oxoglutarate.

The analysis of the functional properties of mitochondria from different sources, combined with the knowledge of their composition, should lead to a better understanding of the interaction between lipids and proteins in membranes and its significance for the characteristic behaviour of these mitochondria.

In previous papers [2—9] we have shown that human liver mitochondria have some particularities and that special care in their isolation and in the study of their enzymic properties is required; it was also shown that human liver mitochondria have a peculiar lipid composition. In this paper we describe some functional properties of human liver mitochondria in comparison with rat liver mitochondria.

## MATERIAL AND METHODS

Human liver mitochondria were isolated by the procedure of Benga *et al.* [8] and rat liver mitochondria as previously described [2]. Oxygen uptake was measured polarographically [11] and spectrophotometrically by the method of Bârzu *et al.* [1], adapted for small quantities of biological materials. ATP synthesis was measured from the amount of glucose-6-phosphate obtained in the presence of hexokinase and glucose [21]. Adenosine-triphosphatase (ATPase) (EC 3.6.1.3.) activity was measured as previously decribed [7]. Malate dehydrogenase (EC 1.1.1.37.), isocitrate dehydrogenase (EC 1.1.1.41.), glutamate dehydrogenase (EC 1.4.1.2.) and 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30.) activities were

determined as described by Hoppel and Cooper [15], monoamine oxidase (EC 1.4.3.4.) after Weisbach *et al.* [25] and Tabor *et al.* [23] and adenylate kinase (EC 2.7.4.3.) after Pedersen [19]. Cytochrome *c* oxidase (EC 1.9.3.1.) activity was followed spectrophotometrically at 550 nm as described by Smith *et al.* [22]. Adenine nucleotides translocation was measured by the inhibitor-stop method, essentially as described by Winkler *et al.* [26]. Mitochondrial protein was determined after Lowry *et al.* [17].

## RESULTS

*The integrity and purity of the mitochondrial fraction.* A typical measurement of the oxygen uptake by the spectrophotometric method is shown in Fig. 1. Respiratory activities of mitochondria isolated from normal human liver are presented in Table 1. As can be seen, the conditions of incubation are of great importance when measuring the respiratory activities of human liver mitochondria. This should be taken into consideration in establishing the reference values of mitochondrial activities for the modifications occurring in diseases. The effect of the hexokinase-glucose system is dependent on the source of mitochondria [16], [24]. With human liver mitochondria, the addition of hexokinase and glucose stimulated, while NaF inhibited the oxygen uptake.

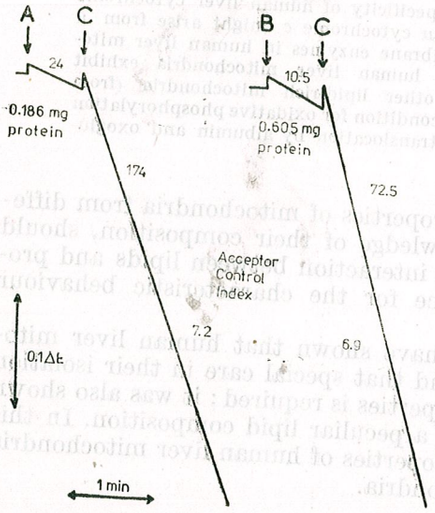


Fig. 1. — Spectrophotometric measurement of oxygen uptake and accepter control index. Human liver mitochondria were incubated at 37°C in the respiratory medium containing in 1 ml final volume : 180 mM sucrose, 50 mM KCl, 16 mM Tris-HCl (pH 7.4), 2.5 mM EDTA, 2.5 mM $MgCl_2$, 5 mM phosphate buffer (pH 7.4), 0.5 mg Hexokinase (1 mg = ca. 3.7 EU), 56 mM glucose, 10 mM succinate (A), 6 mM isocitrate plus 4 mM malate (B) and 0.3 mM ADP (C). The number beside the traces indicates the oxygen uptake expressed as nAtoms/min/mg of protein. Other details of the spectrophotometric measurement of oxygen uptake are as previously described [1], [8].

$Mg^{2+}$ — and dinitrophenol-stimulated ATPases have been used as very sensitive indicators of mitochondrial integrity [7]. $Mg^{2+}$ stimulated the latent ATPase three fold, whereas the stimulation by dinitrophenol is higher (Table 2).



*Functional properties of human liver mitochondria.* Certain peculiarities of the enzymic activities of human liver mitochondria are worth mentioning. Defatted BSA (bovine serum albumin) had a spectacular effect on the 3-hydroxybutyrate dehydrogenase activity of human liver mitochondria, the activity being increased by a factor or two. No significant effect of albumin was noted with the other enzymic activities listed in Table 3. While the specific activities of most of the enzymes are higher in rat that in human liver mitochondria, the activity of isocitrate

*Table 1*

**Respiratory activities of human liver mitochondria**

The oxygen uptake was measured spectrophotometrically [1], [11]. Mitochondria were incubated a 37°C in the respiratory medium containing in 1 ml final volume : 10 mM sucrose, 50 mM KCl, 16 mM Tris-HCl (pH 7.4), 2.5 mM EDTA, 2.5 mM $MgCl_2$, 5 mM phosphate buffer (pH 7.4), 10 mg defatted BSA, 0.04 mEq $HbO_2$. Additions were 0.5 mg hexokinase (NBC, 1 mg = ca. 3.7 EU) plus 56 mM glucose, and 10 mM NaF. Acceptor control index was calculated as the ratio of the respiratory rate in the presence of ADP to the rate before ADP had been added.

| Substrate | Additions Hexokinase plus glucose | NaF | Oxygen uptake (nAtoms/min/mg protein | Acceptor Control Index |
|---|---|---|---|---|
| Succinate (10 mM) | + | − | 134.1±9.9[a] | 6.2±0.6[a] |
| | − | − | 102.6±6.2 | 6.1±0.4 |
| | + | + | 123.0±9.3 | 5.9±0.3 |
| Pyruvate (6 mM), plus malate (4 mM) | + | − | 53.1±5.7 | 5.0±0.6 |
| | − | − | 30.2±4.6 | 4.5±0.5 |
| | + | + | 32.7±6.3 | 4.7±0.4 |
| Isocitrate (6 mM), plus malate (4 mM) | + | − | 60.2±4.7 | 5.6±0.4 |
| | − | − | 46.6±5.0 | 5.0±0.4 |
| | + | + | 50.4±3.5 | 5.1±0.3 |

[a] Mean and standard error of the mean for 8 determinations

*Table 2*

**ATPase activity of mitochondria from rat and human liver mitochondria**

The reaction mixture (final volume 0.2 ml) contained 5 mM ATP, 150 mM sucrose, 50 mM KCl, 50 mM Tris-acetate (pH 8.5), 0.5 mM EDTA and albumin (1% final concentration) ; additions, where indicated were 5 mM $MgCl_2$ and 0.4 mM dinitrophenol. ATPase activity was expressed as nmole Pi/min/mg of protein. The results are the mean ± standard error of the mean for 12 determinations.

| Conditions of assay | Rat liver | Human liver |
|---|---|---|
| Basal activity | 17.0±3.7 | 10.2±2.1 |
| 5 mM $Mg^{2+}$ | 35.7±6.0 | 29.9±5.2 |
| 0.4 mM dinitrophenol | 359.3±6.0 | 162.3±12.5 |

dehydrogenase is higher in humans. Isocitrate was the best NADH-linked respiratory substrate for human liver mitochondria. It was interesting to notice the specificity of human liver cytochrome oxidase towards human cytochrome *c*; rat liver mitochondria oxidized human or horse cytochrome *c* at the same rate, while human liver mitochondria oxidized human cytochrome *c* at a much higher rate than horse cytochrome *c*. This finding is fully discussed elsewhere [3].

*Table 3*

**Enzymic activities of rat and human liver mitochondria**

The activities were measured after a previous solubilization of the organelles with the nonionic detergent Lubrol WX (0.5 mg/mg of protein). The reaction medium of 1 ml volume contained 0.02 — 0.20 mg of mitochondrial protein. The specific activity is expressed as nmoles substrate transformed/min/mg of protein. Other details as indicated under "Material and Methods".

| Enzyme | Human Liver | Rat Liver |
|---|---|---|
| 3-hydroxybutyrate-dehydrogenase : | | |
| without BSA | 33.7±4.1[a](5)[b] | 110±25[a](5)[b] |
| with BSA | 70.5±2.2 (5) | 115±23 (5) |
| Malate-dehydrogenase | 1.692±140 (5) | 3.200±230 (4) |
| Glutamate-dehydrogenase | 27.5±4 0 (5) | — |
| Isocitrate-dehydrogenase [c] | 112.0±20 (7) | 69±12 (6) |
| Monoamine oxidase | 3.9±0.2 (4) | 5.2±0.3 (4) |
| Adenylate-kinase | 614±32 (5) | 1.800±75 (3) |
| Cytochrome oxidase [d] | | |
| with horse cytochrome *c* | 9.1±2.3(14) | 106±21 (5) |
| with human cytochrome *c* | 47.1±7.2(10) | 92±18 (5) |

[a]Mean and standard error of the mean
[b]Number of experiments
[c]The specific activity with NADP as acceptor
[d]The specific activity is calculated from the first order reaction velocity constant ($min^{-1}$. mg $protein^{-1}$. ml)

Taking into account the high lipid content of human liver mitochondria it seems reasonable that they might exhibit certain functional properties similar to other lipid-rich mitochondria, and this appears to be the case. The concentration of EDTA required to yield a maximal P/O ratio was found to depend on the $Mg^{2+}$ concentration and — as for rat brown adipose tissue mitochondria [14] — it must be nearly equal to that of $Mg^{2+}$ (Fig. 2). It was interesting to find that albumin and α-oxoglutarate stimulated adenine nucleotide translocation in human liver mitochondria (Table 4) as described for mitochondria from brown adipose tissue of guinea pigs and rats [10].



Fig. 2. — Influence of $Mg^{2+}$ and EDTA on phosphorylation of human liver mitochondria. The incubation medium (1 ml final volume) contained: 180 mM sucrose, 50 mM KCl, 16 mM Tris-HCl (pH 7.4), 10 mM glutamate, 10 mM NaF, 20 mM glucose, 2 mg yeast hexokinase (3.7 EU/mg), 10 mg BSA, 0,1 mM ADP. The reaction was initiated by addition of 0.3 mg of mitochondrial protein. After 10 min of incubation at 37°C, 0.5 ml of 1.5 N perchloric acid was added. The glucose-6-phosphate was measured enzymatically in the neutralized extract, by examining the reduction of NADP, with glucose-6-phosphate dehydrogenase at 340 nm.

Ordinate: nmoles ATP/min/mg protein

Abscissa: [$MgCl_2$], mM.

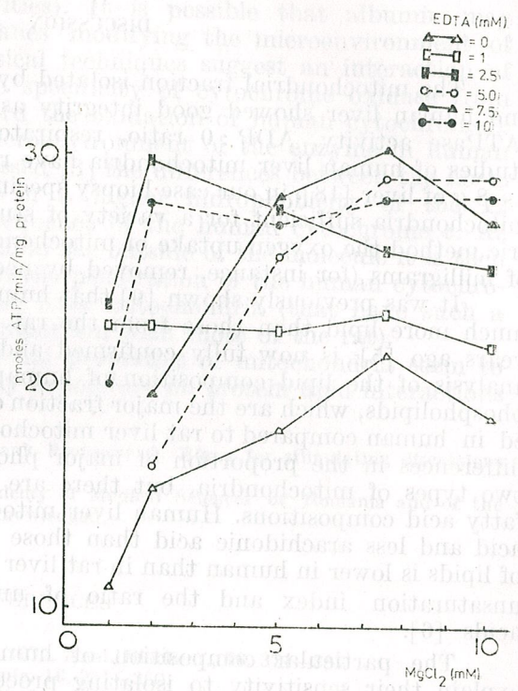


*Table 4*

**Effect of serum albumin and oxoglutarate on ADP translocation in human liver mitochondria**

The translocation was measured at 0°C in 240 mM KCl, 40 mM Tris-HCl, pH 7.4, 2 mM EDTA. The reaction was initiated by addition of 60 μm, [$^{14}C$] ADP and stopped after 5 min with 60 μM atractyloside and dilution with 5 ml ice-cold 250 mM sucrose. The mitochondria were isolated by Millipore filtration, washed with 120 mM KCl, 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, then dissolved and counted for radioactivity. The results are the means of 4 experiments.

| Additions during preincubation | ADP translocated (nmole/mg protein) |
|---|---|
| None | 10.4 |
| oxoglutarate (5 mM | 25.9 |
| Albumin (1%) | 33.5 |

## DISCUSSION

The mitochondrial fraction isolated by our procedure from both rat and human liver showed good integrity as judged by biochemical tests (ATPase activity, ADP : O ratio, respiratory control). Whilst previous studies of human liver mitochondria have required biopsies ranging from 4—8 g of liver [18], in our case biopsy specimens of less than 0.5 g yielded mitochondria sufficient for a variety of studies. By the spectrophotometric method the oxygen uptake of mitochondria from samples in the range of milligrams (for instance, removed by needle biopsy) could be studied.

It was previously shown [9] that human liver mitochondria contain much more lipid than those from the rat. This finding, noticed several years ago [5], is now fully confirmed and completed with the detailed analysis of the lipid composition of human liver mitochondria [6]. The phospholipids, which are the major fraction of membrane lipids, are increased in human compared to rat liver mitochondria. There are no significant differences in the proportion of major phospholipid classes between the two types of mitochondria, but there are differences in the constituent fatty acid compositions. Human liver mitochondria contain more linoleic acid and less arachidonic acid than those of the rat. The unsaturation of lipids is lower in human than in rat liver mitochondria as judged by the unsaturation index and the ratio of unsaturated to saturated fatty acids [6].

The particular composition of human liver mitochondria might explain their sensitivity to isolating procedures [8], as well as certain functional peculiarities. It was interesting to see that they exhibit functional properties similar to mitochondria from rat brown adipose tissue (which are also lipid-rich), like the effects of different concentrations of $Mg^{2+}$ and EDTA on oxidative phosphorylation and the stimulation of adenine nucleotide translocation by albumin. The stimulation of adenine nucleotide translocation by oxoglutarate was related by Christiansen *et al.* [10] to the increase of the exchangeable nucleotides ATP and ADP at the expense of AMP, while the effect of albumin was at the true rate of the translocation.

It is well known that many mitochondrial enzymes are phospholipid-dependent [12]. One of the first demonstrations of a lipid requirement for activity of an isolated enzyme was the report that mitochondrial 3-hydroxybutyrate dehydrogenase required phosphatidylcholine for activity [20]. Recent studies on lipid specificity of 3-hydroxybutyrate dehydrogenase activation using a variety of synthetic phospholipids [13] have shown that 3-hydroxybutyrate dehydrogenase has an unusual specificity for both the ionic groups and the fatty acid side chains. The activity of human liver mitochondrial 3-hydroxybutyrate dehydrogenase was markedly stimulated by BSA. Since the free fatty acid content of human liver mitochondria isolated in the presence of BSA was not too different from that of rat liver mitochondria, the effect of BSA cannot be explained only by the binding of free fatty acids (the usual explanation for the effects



of BSA on mitochondrial activities). It is possible that albumin may interact directly with membranes modifying the microenvironment of enzymes. Recent data using physical techniques suggest an interaction of BSA with membranes [4]. The specificity of cytochrome oxidase from human liver mitochondria toward the oxidation of human cytochrome *c* might also be related to the microenvironment of the enzyme in human membranes. As previously discussed [3] the differences between the horse and human cytochrome *c* reside in a higher hidrophobicity of the 12 replacements in the amino acid sequence of the human cytochrome *c*. As all the 12 replacements are found on the outside of the molecule it is then possible that they are involved in the penetration of the human cytochrome *c* in the membrane of human liver mitochondria (that have such a different lipid composition in comparison with those of the rat).

In conclusion, some functional properties of mitochondria seem to be species related and particularly dependent on protein lipid interactions in mitochondrial membranes.

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# THE HYPOLIPEMIANT AND HYPOCHOLESTEROLEMIANT ACTION OF NYSTATIN AND CM NYSTATIN

BY

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The action of nystatin and of CM nystatin given i.m. in doses of 0.666 mg/kg b.wt./day and 25 mg/kg b.wt./day, respectively, on lipemia and cholesterolemia have been investigated in laboratory animals (Chinchilla rabbits) fed a mixed atherogenic regimen (1.55 g cholesterol/kg b.wt./day for two weeks 0.355 g cholesterol/kg b.wt./day for five weeks and 0.765 g cholesterol/kg b.wt./day for five weeks). Important hypolipemiant and hypocholesterolemiant actions of these antibiotics have been evidenced, nystatin being more efficient than CM nystatin.

The importance of experimental investigations in laboratory animals of new drugs with hypocholesterolemiant and hypolipemiant action has been pointed out by several workers and has a very clear practical motivation [15], [16].

In the present paper we studied, in this respect, two polyenic antibiotics : nystatin and CM nystatin, products manufactured by ICCFCCA — Iași under patent RSR no. 71274, 1979 [20].

We had in mind the fact mentioned in the literature [5] [10], [11], [16], [17] that the penetration of polyenes of biosythesis in the cells is based on their specific interaction with the membrane cholesterol, interaction which is antagonized by the free cholesterol in the medium [12], [13] being simultaneously influenced by the value of the cholesterol phospholipid ratio in the membrane structure [4].

However, there are some brief observations regarding a hypocholesterolemiant effect of some polyenes in small laboratory animals [18], [21], the authors showing that "this property has not yet been exploited" for practical applications.

In one of our previous works we also noticed that the semisynthetic product CM nystatin has an obvious capacity of interacting with the cholesterol at the level of cell membranes, leading to its elimination from the structure.

All these observations conduced us to the idea of some possible interactions of polyenes with cholesterol in the blood or deposited on the vascular walls. This is why we considered worth while to investigate if the drugs mentioned above had important hypocholesterolemiant and hypolipemiant properties, which might eventually be used as therapeutic agents.

With these in mind, we carried out a series of chronic experiments on animals in which, by procedures described in literature [1], [3], [7], [8], [19] experimental atherosclerosis was produced, simultaneously following the effects of nystatin and CM nystatin on the mode of its installation expressed by the evolution of the level of blood total lipids and cholesterol.

## MATERIAL AND METHODS

The experiments were performed on rabbits (Chinchilla) of the same age and with a body weight around 2 kg at the beginning of the experiment. The animals were divided into three groups (4 — 6 animals each) and fed an atherogenic regimen with no antibiotic, supplemented with nystatin or CM nystatin, respectively.

We chose a mixed atherogenic regimen [7] giving the cholesterol in various amounts at different stages : in the first two weeks (heavy regimen) each animal received 1.55 g cholesterol/kg b.wt./day, in the next five weeks (light regimen) 0.355 g cholesterol/kg b.wt./day, and in the last five weeks (medium regimen) 0.765 g cholesterol/kg b.wt./day. The source of cholesterol was the dry, ground yolk, in which its concentration was determined [6], [9].

Each time, and for every type of regimen, the amount of yolk was fed in two equal portions per day, its ingestion being strictly controlled.

The polyenes studied have been administred by i.m. injections. For CM nystatin a dose of 25 mg/kg b.wt./day in one ml saline in one shot was used, whereas for nystatin a dose of 0.666 mg/kg b.wt./day in 1 ml 1,2-propylen glycol in one injection. The dose of CM nystatin was established according to the dose used in humans by other authors, whereas for nystatin which has much more pronounced effects on cell membranes the dose was inserred from the ratio of the two substances which had equal effects on the membrane. The polyenes were administered for the entire duration of the experiments.

In order to study the installation of hyperlipemia and hypercholesterolemia provoked by the atherogenic regimen, as well as the effects of polyenes on these blood parameters, the animals were bled and the serum concentrations of lipids and cholesterol [9] [22] before the treatment and after, at 2,4,5,7,9,5 and 12 weeks, were analyzed.

## RESULTS

### A. TOTAL SERUM LIPIDS

a. The determinations of total lipids in the serum of animals subjected to the atherogenic regimen, but untreated with polyenes (Group I) showed a gradual instalation of a pronounced hyperlipemia (Fig. 1 — I, L).

After two weeks of heavy regimen, the lipid concentration increased by 7.5 times (2393.75 mg %) compared to the normal. During the light regimen, a slight decrease was observed compared to the value registered previously, then the lipids increased abruptly again, reaching a concentration of 4620 mg% at the end of the medium regimen (14.66 times higher than normal).



b. Treatment with CM nystatin of animals maintained under atherogenic regimen (Group II) had a significant influence on the increase of total serum lipids (Fig. 1— II, L). After 2 weeks of heavy regimen their value was only 6.46 times higher (1690 mg %) than the initial value, during

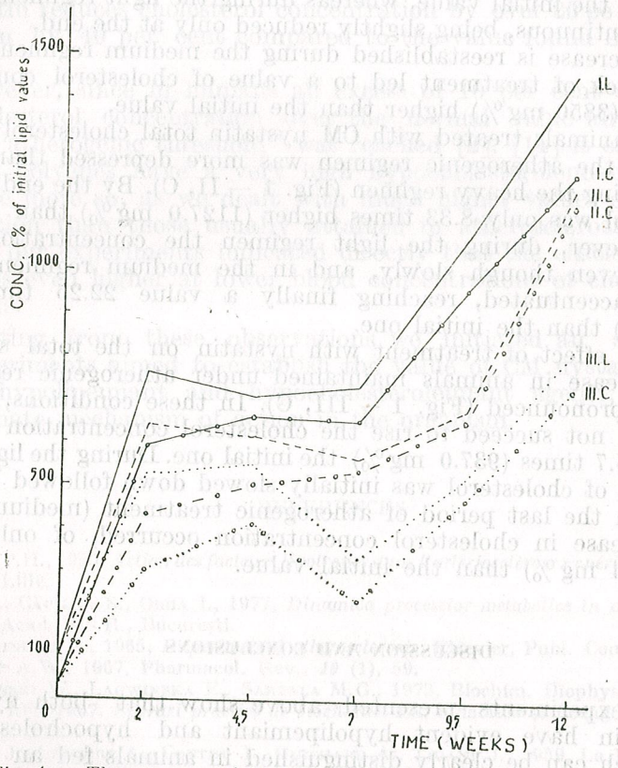


Fig. 1. — The serum variation of total lipids (L) and cholesterol (C), in animals treated with an atherogenic regimen without antibiotic (I), with CM nystatin (II) or nystatin (III).

the light regimen a slightly more pronounced decrease than in controls was noticed, whereas during the medium regimen the increase in concentration resulted at the end of the treatment in a serum lipid value only 12 times higher (3137.50 mg %) than the initial one.

c. A stronger influence on the total serum lipid increment was observed after treating (Group III) the animals fed the atherogenic regimen with nystatin (Fig. 1 — III, L). After 2 weeks of heavy regimen the lipids increased only 5.2 times (1637.50 mg %) the initial value. During the light regimen the lipid concentration decreased very much compared to the level reached in the previous stage, and the increase registered during the medium regimen led in the end to a lipid concentration of only 2562.5 mg %.

B. TOTAL SERUM CHOLESTEROL

a. The increase of total cholesterol in the serum of animals fed the atherogenic regimen and untreated with polyenes displayed a curve similar to that of total lipid increment (Fig. 1 — I, C). After 2 weeks of heavy regimen the cholesterol concentration increased 12.8 times (1862.5 mg%) compared to the initial value, whereas during the light regimen the increment was continuous, being slightly reduced only at the end.

The increase is reestablished during the medium regimen and after the 12th week of treatment led to a value of cholesterol concentration 26.46 times (3850 mg%) higher than the initial value.

b. In animals treated with CM nystatin total cholesterol increment induced by the atherogenic regimen was more depressed than the lipid increase during the heavy regimen (Fig. 1 — II, C). By the end, the value of cholesterol was only 8.33 times higher (1127.0 mg%) than the initial value. However, during the light regimen the concentration increase continued, even though slowly, and in the medium regimen period it was again accentuated, reaching finally a value 22.25 times higher (3010 mg%) than the initial one.

c. The effect of treatment with nystatin on the total serum cholesterol increase in animals maintained under atherogenic regimen was even more pronounced (Fig. 1 — III, C). In these conditions, the heavy regimen did not succeed to rise the cholesterol concentration to a value more than 5.7 times (937.0 mg%) the initial one. During the light regimen the increase of cholesterol was initially slowed down followed by a sharp decrease. In the last period of atherogenic treatment (medium regimen) a new increase in cholesterol concentration occurred, of only 14 times higher (2284 mg%) than the initial value.

## DISCUSSION AND CONCLUSIONS

The experiments presented above show that both nystatin and MC nystatin have evident hypolipemiant and hypocholesterolemiant effects which can be clearly distinguished in animals fed an atherogenic regimen.

More efficient, from this point of view, is nystatin, which influences both total lipid and cholesterol concentrations in the serum such that at the end of the experiment their values are only a little over the half-value of control animals.

The efficiency of these two polyenes increases at a lower blood loading with lipids and cholesterol (see the situation during the light regimen period).

The depressing effect of CM nystatin on total lipid increase influences much stronger other lipid fractions than the cholesterol, so that, on the one hand, during the light regimen period the decrease of total lipids is accompanied by an increase of cholesterol, and on the other hand, in the following period, the cholesterol concentration varies only slightly compared to total lipid concentration.



However, nystatin influences more uniformly both the cholesterol and the other serum lipids, so that during the entire period of atherogenic treatment their ratio is more stable.

The efficiency of the drugs studied by us may be quantitatively evaluated, although indirecty, based on the reduction imposed on the blood cholesterol when this reaches a maximal concentration (at the end of the treatment). Therefore, it is evident that, in these conditions CM nystatin reduces cholesterol concentration by over 20 pe rcent, whereas nystatin by 40 per cent compared to the value found in nontreated animals.

However, since in humans an excess of 10 per cent of the blood total cholesterol concentration over the normal value already means that the "atherogenic threshold" was reached [2], [14] it can be stated that both polyenes have a very high hypocholesterolemiant efficiency. This is the more so, as we dealt with much higher values of cholesterol concentration than those usually attained in atherosclerosis. Moreover, as shown, our experiments indicated directly that the efficiency of these polyenes is even higher at lower blood concentrations of cholesterol and total lipids.

Starting from these observations we initiated an aggregate of other experiments aimed to establish the value of CM nystatin and nystatin as hypolipemiant and hypocholesterolemiant agents, as well as their intimate mechanism of action in the organism.

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# ON THE CELL MEMBRANE REACTIVITY AND ITS BASIC MECHANISMS

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It is shown that the biological membrane is an adaptative structure which ensures a complex and accurate reactivity. The independent reaction ability of membrane layers allows a discrimination between the action of external and internal factors. The differentiation of the structural elements of each layer according to the specific equilibrium with the ions in the own adjacent phase ensures differentiated reactions when an increasing or a decreasing variation of essential ion ratio ($K^+$:$Ca^{2+}$ ratio for the external layer) takes place. The differentiation of the elements of the same structuring type according to their degree of sensitivity toward destructuring ion and the number corresponding to each degree, ensures differential reactions according to the nature of the ion which determines the ionic ratio variation and the extent of its value.

Investigations over the last years have demonstrated more and more clearly that the cell membrane does not respond to the variation of extracellular and intracellular factors in a more or less passive way as is the case of physico-chemical membranes [5], [19], [20], [22]. The structural and functional properties of the biological membrane depend on the molecular and supramolecular organization of its components. The membrane is neither homogeneous, nor static, but presents a pronounced asymmetry, being modified in terms of the complex interactions which are established with the adjacent phases.

In one of our previous papers [5], we emphasized on the experimental data which led us to the conclusion that each layer of the membrane has the possibility of independent changes based on their own interactions with the corresponding adjacent phase. More recently some other authors have adopted this point of view [19] bringing new experimental arguments in its favour.

The ability of an independent behaviour of membrane layers regarding bioelectric and selective permeability properties does not, however, rule out the possibility of some complex responses of the membrane in which the phenomena occurring in the two layers of the membrane are strictly correlated. This is so, for example, in the automatic development of excitation process ensured by feedback connections between membrane layers [7].

The passive modifications of the membrane potential (depolarizations and hyperpolarizations) as well as the "passive" permeability changes are, however, the result of some phenomena occurring at the level of one layer of the membrane, without a direct and essential involvement of the other layer.

This specific feature of the biological membrane response to the fluctuations of internal and external conditions explains, at least partially, the great variety and complexity of the phenomena occurring at its level.

We have stated previously [5], [6] that the membrane components playing the main role in bioelectrical and permeability phenomena are the phospholipids. Their complex modalities of supramolecular organization, multiple possibilities of conformational and phase transition modifications [14], [20], [21], [24], as well as their ion exchange properties [16], [23] have been lately increasingly clearly demonstrated. Some other membrane models, which admitted more or less fermly that the phospholipids play an essential role in membrane processes [1], [2], [3], [20] have also been elaborated.

The idea of electrical mosaic and fixed charge asymmetry on the two sides of the membrane, re-evaluated more and more often by several authors [3], [11], [12], [19], [22], was developed by us in the sense that there is a prevalence of fixed negative charges (and therefore of the cationitic properties) in the external layer of the membrane where there are also positive fixed charges (anionitic properties) and a prevalence of the fixed positive charges (and therefore of anionitic properties) in the internal layer of the membrane where fixed negative charges (cationitic properties) are also present. Thus, each layer of the membrane has an electric mosaic and also its own and characteristic electrical potential.

We explained the bioelectrical properties as essentially derived from the characteristic charge of phospholipidic micellae according to their ion exchange properties, and a set of experimental data [5], [6], [19], [20], [22] indicated a parallelism between the number of fixed negative charges and the number of structural formations closely packed (laminar micellae) on the one hand, and between the number of fixed positive charges and the number of less packed formations (globular micellae) on the other hand, in each of the membrane.

On this basis, we characterized and correlated some of the bioelectrical properties with those of permeability characteristic of each layer, and also these own properties in relation to their specific structural basis.

Considering the membrane quantitative composition in various phospholipids [13], on the one hand, and their specific supramolecular organization (micellar), in normal resting conditions [20] on the other hand, we concluded that in the membrane external layer the laminar micellae are structurated by the Ca ions, whereas the globular micellae by K ions. In favour of this statement are the arguments presented by H. A. Kolb and G. Adam [19], who distinguished some "open" cation chanals with regulation loci occupyed by two alkaline ions ($K^+$) and some "closed" cation chanals with a locus occupied by a bivalent cation ($Ca^{2+}$).

However, the organization specific to each layer, described above, allowed only the explanation of the membrane properties during its normal resting state, but not of the bioelectrical and permeability modifications featuring other stationary states, as those of passive depolarization or hyperpolarization.

Some time ago, several experimental observations showed, however, that the normal resting state of the membrane (and more recently it



was also demonstrated that in fact, the normal state of its external layer), essentially depends on the ratio between $K^+$ and $Ca^+$ concentrations in the extracellular phase [5], [19], [22].

The modification of the normal $K^+ : Ca^{2+}$ ratio in the external medium, toward its increment (either by an increase of $K^+$ concentration, or by a decrease of $Ca^{2+}$ concentration) results in a depolarization of the membrane. In each of these situations, $K^+$ exceeding $Ca^{2+}$ acts, at the level of the external layer, as a destructuring ion on the laminar phospholipidic micellae (closed, cationitic micellae), determining their phase transition to globular micellae. However, the depolarization produced by $K^+$ increase is quickly installed and may have high magnitudes whereas that induced by $Ca^{2+}$ concentration decrease occurs slowly and has relatively reduced amplitudes.

When the normal $K^+ : Ca^{2+}$ ratio in the extracellular medium is modified toward its decrement (either by decrease of $K^+$ concentration or by $Ca^{2+}$ concentration increase) a hyperpolarization of the membrane occurs. In these situations $Ca^{2+}$ becomes in excess compared to $K^+$ and acts as a destructuring ion on the globular micellae (open, anionitic micellae) of the external layer, determining their phase transition to laminar micellae [5], [19], [20], [23], [24]. However, the hyperpolarization induced by the increase of external $Ca^{2+}$ takes place rapidly and may exhibit high amplitudes, whereas the hyperpolarization brought about by external $K^+$ decrease is slowly installed and has reduced amplitudes.

Similarly, the membrane internal layer also modifies specifically its electrical and permeability state as a response to the variation, in one direction or the other, of the intracellular factors involved in the structuring or destructuring of its different component phospholipidic formations.

In other works [8], [9], [10] we showed that there is no solid base, either theoretical, or experimental, for considering the behaviour of the biological membrane as being similar to a $K^+$ electrode.

The observations made during the experiments of membrane depolarization by high external $K^+$, which indicated a linear correlation between the membrane potential and the logarithm of $K^+$ external concentration, represented a basis for such interpretations [4], [15], [18].

However, we discussed that the use of Nernst's equation for the concentration cell potential in order to prove a concordance between the values experimentally recorded and those obtained by calculation, is not proper. Such an approach to the problem would rather impose the conclusion that the membrane has no electrical charge for the entire range of $K^+$ external concentrations, which could hardly be admitted.

On the basis of our own concept of the membrane, we interpreted the experimental results of membrane depolarization by high external $K^+$ quite differently [5], [10], [17]. Indeed, it has been observed that when an increase of external $K^+$ by successive equal rates occurs, a decrease of membrane potential by smaller and smaller rates results, which demonstrates a very important fact related to the membrane reactivity (in our case, the reactivity of the external layer). This development of the phenomenon shows that the micellar structures, in general (in our

case, the laminar structures) are differentiated according to their sensitivity toward the characteristic destructuring agent (in this case, $K^+$).

Besides, the qualitative aspect expressed by the fact that some laminar micellae are involved in phase transition even by less higher concentrations of external $K^+$ and other sonly by increasingly higher concentrations, a quantitative aspect of the differentiation of same type micellae is revealed. There are several laminar micellae presentingly a great sensitivity toward the ion with transitional action (in our case $K^+$), and which bind weaker the specific structuring ion (in this case $Ca^{2+}$) and less and less micellae with lower and lower sensitivity, which bind stronger and stronger the structuring ion.

The experimental data allow us to extend these conclusions to the globular micellae of the external layer (membrane hyperpolarization) as well as to both types of micellae of the internal layer [5] (depolarizations and hyperpolarizations internally determined).

Such a qualitative and quantitative differentiation of membrane structures ensures gradual responses of the membrane according to the intensity of the factors acting on it (the value of $K^+ : Ca^{2+}$ ratio), but also differentiated according to the agent actually varying : rapid reaction to the ratio increase by the $K^+$ concentration rise and slow reaction to its increase by $Ca^{2+}$ concentration decrease. However, it offers a modality of self-protection by exposing some additional ever more reduced zones to the high intensity action of these factors.

The arguments presented above show that, as far as its basic reactivity is concerned, the biological membrane is organized and acts as a functionally-adaptive living structure, endowed with numerous and various possibilities of response (and therefore of equilibration with) the variations of factors "outside" it. Thus, the membrane discriminates between the intracellular and extracellular factors, between the increasing and decreasing variations of the ionic ratio, between the constant or variable term of this ratio, as well as between its various quantitative values. The membrane structure thus ensures the possibility of a net discrimination, efficiency of the reaction and self-protection conditions.

All the considerations on the bioelectrical and selective permeability phenomena should take into account the permanent adjustment of the biological membrane properties to the surrounding conditions.

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# PRÉSENCE DE QUELQUES ESPÈCES MÉDITERRANÉENNES DANS LE ZOOPLANCTON DE LA MER NOIRE

PAR

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The author points out for the first time the presence off coast the Romanian continental shelf of the Black Sea of 16 zooplanktonic species newly imigrated from the Mediterranean basin. The distribution in time (November-December 1978, March-April 1979) as well as in the water mass of the mentioned species is analysed in the paper.

L'écosystème pélagique près du littoral roumain a subi durant les années de la 8e décennie une série de transformations importantes d'ordre qualitatif, par rapport à la période antérieure. En dépit d'une faible tendance de réduction de la quantité annuelle d'eaux douces emportés par le Danube, la croissance de leur stock en substances nutritives ou polluantes, à côté de celui des effluents d'origine ménagère et industrielle rejetés dans la zone littorale ont déterminé l'eutrophication accentuée des eaux [4], [5], [7].

L'une des conséquences de ce fait a été le développement explosif du phytoplancton, particulièrement de ses composantes hétérotrophes, marqué au moins durant la saison chaude par des périodes de « floraison » [1].

En ce qui concerne le zooplancton, de telles modifications se sont manifestées aussi, d'une part par la diminution des populations des unes des espèces dominantes, de l'autre, par le développement abondant des autres, dont les nouvelles conditions représentent le facteur stimulateur.

La réduction accentuée durant les dernières années du nombre d'individus appartenant aux espèces des copépodes neustoniques (*Centropages ponticus* Karavaj., *Anomalocera patersoni* Templeton), les fluctuation des populations de *Penilia avirostris* Dana, toutes ensemble à la présence des riches générations pour *Acartia clausi* Giesbrecht, sont quelques-unes des conséquences d'ordre biologique déterminés par l'influence des nouvelles conditions.

Les transformations dans la structure spécifique du zooplancton ne s'arrêtent pas à celles mentionnées auparavant. Il s'agit de l'apparition des nouvelles espèces, dont la présence en Mer Noire ne peut pas être attribuée à l'eutrophication.

Durant l'automne et l'hiver 1978 et le printemps 1979 l'Institut Roumain de Recherches Marines a organisé des recherches complexes en

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mer Noire, dans les eaux du large de la plate-forme continentale. Dans quelques-unes des prises zooplanctoniques prélevées pendant les campagnes de recherches effectuées, on a identifié, outre les formes pontiques communes, la présence de 16 nouveaux taxons se rattachant au plancton du bassin méditerranéen. Le phénomène est d'autant plus intéressant que l'existence de ces organismes dans les eaux de la plate-forme continentale roumaine n'a pas été mise en évidence jusqu'à présent.

Dans cinq des stations faites pendant la campagne de 9 à 15 novembre 1978 (les stations 2, 9, 10, 11 et 12), (latitudes comprises entre 43°59' — 44°53' ; longitudes entre 29°08' — 30°38'), au-dessus des profondeurs de 40 — 112 m, on a rencontré les espèces suivantes :

*Calanus tenuicornis* Dana, 1 copépodite V^e stade
*Calanus gracilis* (?) Dana, 2 femelles, 1 mâle
*Phaenna spinifera* Claus, 1 mâle
*Mecynocera clausi* J.C. Thompson, 1 femelle
*Paracalanus aculeatus* Giesbrecht, 1 femelle
*Paracalanus nanus* G.O. Sars, 1 femelle
*Calocalanus plumulosus* Claus, 1 femelle
*Calocalanus pavo* Dana, 2 femelles
*Calusocalanus arcuicornis* Dana, 6 mâles et 3 copépodites V^e stade
*Oncaea mediterranea* Claus, 1 femelle
*Ctenocalanus vanus* Giebsbrecht, 5 mâles
*Stylocheiron* sp. (larve Furcilia), 1 exemplaire
*Sagitta* sp., 1 exemplaire

Dans la station N° 18, faite au mois de décembre sur 44°01' de latitude et 29°55' de longitude, au-dessus de la profondeur de 85 m on a déterminé :

*Eudoxoides spiralis* Bigelow, 1 exemplaire
*Eucalanus* sp., 1 copépodite III^e stade et 2 femelles de *Paracalanus nanus.*

La présence des espèces méditerranéennes s'est avérée plus faible durant la campagne de 23—31 mars 1979. Dans deux stations seulement (N^os 6 et 7) (44°15' et 44°30', respectivement ; 29°15' et 30°45') on a rencontré une femelle appartenant au *Clausocalanus arcuicornis* et une autre à *Labidocera brunescens* Czern. Bien que cette dernière fut signalée auparavant pour le zooplancton pontique, elle s'y rencontre assez rarement. C'est pour cela qu'on suppose qu'elle est venue avec les précédentes.

À une seule exception (la larve d'Euphausides), toutes les organismes ont été trouvés en bon état, ce qui dénote qu'au moment de la fixation de la prise elles furent vivantes.

L'analyse de la distribution verticale des nouvelles espèces (tabl. 1) met en évidence leur présence dans les niveaux profonds de l'eau (50—75 m), mais aussi près de la surface (0—10 m ; 10—25 m). Il faut aussi mentionner que la plupart des espèces a été rencontrée pendant la saison d'automne-commencement de l'hiver, intervalle qui correspond au moindre débit du Danube, quand, probablement par compensation, le courant profond des eaux méditerranéennes vers la mer Noire s'intensifie.

La pénétration des espèces du bassin méditerranéen en mer Noire n'est pas un phénomène nouveau. Nous avons déjà apporté des arguments



*Tableau 1*

Distribution verticale des espèces méditerranéennes dans les eaux de la mer Noire (exemplaires)

| Espèces \ Horizons | Stations 2 | 6 | 7 | 9 | 10 | 11 | 12 | 18 |
|---|---|---|---|---|---|---|---|---|
| | 10—0 | 25—10 | 10—0 | 25—10 | 50—25 | 75—50 | 100—75 | 50—25 | 75—50 | 10—0 | 25—10 | 25—10 | 50—25 |
| *Calanus tenuicornis* Dana | | | | | | 1 | | | 1 | 3 | | | 1 |
| *Calanus gracilis* (?) Dana | | | | | | | | | 1 | | | | |
| *Eucalanus* sp. | | | | | | | | | | | 1 | | 2 |
| *Mecynocera clausi* J.C. Thomps. | | | | | | | | | | 1 | | | |
| *Paracalanus aculeatus* Giesbr. | | | | 1 | | | | | | | | | |
| *Paracalanus nanus* G.O. Sars | 1 | | | 1 | | | | | | | | | |
| *Calocalanus plumulosus* Claus | | | | | | | 2 | | | | | | |
| *Calocalanus pavo* Dana | 2 | | | | | | 7 | | | | | | |
| *Clausocalanus arcuicornis* Dana | | | 1 | | | | | | | 1 | | | |
| *Ctenocalanus vanus* Giesbr. | | 5 | | | | | 1 | 1 | | | | | |
| *Labidocera brunescens* Czern. | | | | | | | | 1 | | | | | |
| *Phaenna spinifera* Claus | | | | | | | | | | | | | 1 |
| *Oncaea mediterranea* Claus | | | | | | | | | | | | | |
| *Stylocheiron* sp. | | | | | | | | | | | | | |
| *Sagitta* sp. | | | | | | | | | | | | | |
| *Eudoxoides spiralis* Bigelow | | | | | | | | | | | | | |

sur son existence quand on a mis en évidence la présence des larves de Cirripèdes pédonculés dans les eaux peu profondes au voisinage de la Station Agigea [7]. Les chercheurs soviétiques ont signalé leur présence dans les eaux prébosphoriques et dans celles de la Crimée, mais aussi du golfe de Sébastopol [2], [3], [6].

L'arrivée des organismes méditeranéens dans les eaux de la plateforme continentale de la mer Noire représente une nouvelle preuve pour le phénomène de méditerranisation continue de la faune du bassin. Même si ces espèces furent rencontrées par peu d'individus, leur présence en Mer Noire indique une tendance de conquérir ce milieu aussi.

Parmi celles-ci les espèces euryhalines survivent et sans doute elles commencent à s'acclimater aux nouvelles conditions. Leur mention dans la partie NW du bassin où l'influence du Danube devrait se ressentir à un plus haut degré, plaide aussi pour le fait que le phénomène de méditerranisation de la faune pontique embrasse un aréal de plus en plus grand, étant favorisé par les changements des conditions de la mer Noire.

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# L'EFFET DE FERTILISATION CHIMIQUE D'UNE PRAIRIE NATURELLE SUR DES POPULATIONS D'ORIBATES *(ACARINA, ORIBATEI)*

PAR

N. VASILIU et MAGDA CĂLUGĂR

The authors discuss the influence of a first year of fertilization with fertilizers based on P, K, N on the *Oribatids* community structure in a *Agrostis tenuis* and *Festuca rubra* pasture (Suceava county : Romania). It was shown that the position of the species regarding their dominance relationship is not affected. In the case of $P_{83}$, $K_{108}$, $N_{180}$ doses appear small fluctuations in the cenotic stability.

La fertilisation chimique d'une prairie permanente avec de grandes doses d'engrais azoteux, longtemps appliqués a comme résultat la réduction de la diversité des populations d'*Oribates* et le changement du rapport des forces entre des espèces (Vasiliu N., Călugăr M., 1978), [14], [15].

Nos recherches se sont proposé de surprendre les éventuelles modifications qui apparaissent dans la structure et la stabilité de la communauté d'*Oribates* adultes dans les premières étapes de l'application des engrais chimiques.

## LES CARACTÉRISTIQUES DU BIOTOPE

Les recherches ont été entreprises dans un champ expérimental aménagé en 1979, dans une prairie permanente de *Festuceto-Agrostetum tenuis montanum* Csürös et Resmeriţă, 1960, appartenant à la commune Ciprian Porumbescu dans le point nommé Bălăceana, de la région de Suceava (alt. 460 m).

La fertilisation a été réalisée avec de l'azotate d'ammonium, du superphosphate et avec du sel potassique (tabl. 1). Les quantités d'engrais chimiques calculées en kilos de substance active à l'hectare ont été appliquées différemment en variantes (Popovici D., Ciubotaru C., 1979).

Les conditions climatiques de l'intervalle étudié (avril-septembre)se sont catactérisées par un printemps précoce avec des périodes de froid, un été à valeurs thermiques élevés en juin mais baisses dans les autres mois et en général excédentaire du point de vue pluviométrique (Davidescu G., 1979).

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*Tableau 1*

**Le schéma de l'administration des engrais minéraux (les quantités sont exprimées dans kilos substance active à l'hectare)**

| Variante | Intégral | | Intégral ou fractionné | | |
|---|---|---|---|---|---|
| | P | K | N | | |
| | printemps | printemps | printemps | été | automne |
| Témoin | – | – | – | – | – |
| $N_{120}$ | 55 | 72 | 120 | – | – |
| $N_{180}$ | 83 | 108 | 180 | – | – |
| $N_{60\times2}$ | 55 | 72 | 60 | 60 | – |
| $N_{60\times3}$ | 83 | 108 | 60 | 60 | 60 |

## MATERIEL ET MÉTHODES

On a prélevé 240 (16 variante × 5 variantes × 3 temps de récolte) épreuves de sol de l'herbage à l'aide de la sonde Mac Fayden (volume : 40,69 cc).

Les dates du prélevement : le 25 mai, le 15 juillet et le 5 septembre 1979.

On a mis en évidence la structure de la communauté d'*Oribates* à l'aide des indices suivants : la constance ou la continuité de l'apparition — C, la densité relative — D.r., l'indice de signification écologique — W (Djuba, 1958) ; la diversité — H(S) (Shannon-Wiener, 1949) et l'équitabilité — ε (Lloyd, Ghelardi, 1964) [2], [3], [4], [5], [6], [7], [9], [10], [11], [12]. Les espèces où ont prédominé les valeurs de l'indice de signification écologique dépassant 5% ont été considérées comme étant des éléments édificateurs pour la communauté ; les autres espèces, qui ont enregistré des valeurs sous 5% représentent des éléments accompagnateurs.

L'affinité cénotique entre le témoin et les variantes et entre les variantes, a été établie grâce à l'analyse de la corrélation de rang (le coefficient Spearman, 1904) [13].

## RÉSULTATS

On a identifié 21 espèces d'*Oribates* : 1. *Brachychthonius berlesei* Willmann, 1928 ; 2. *Platynothrus peltifer* (C.L. Koch, 1840) ; 3. *Malaconothrus gracilis* van der Hammen, 1952 ; 4. *Ceratoppia quadridentata* (Haller, 1880) ; 5. *Tectocepheus velatus* Michael, 1880 ; 6. *Oppia insculpta* Paoli, 1908 ; 7. *Oppia subpectinata* (Oudemans, 1901) ; 8. *Oppiella nova* (Oudemans, 1901) ; 9. *Quadroppia quadricarinata* (Oudemans, 1916) ; 10. *Suctobelbella subtrigona* (Oudemans, 1916) ; 11. *Scheloribates laevigatus* (C.L. Koch, 1836) ; 12. *Liebstadia similis* (Michael, 1888) ; 13. *Haplozetes vindobonensis* Willmann, 1935 ; 14. *Trichoribates trimaculatus* (C.L. Koch,



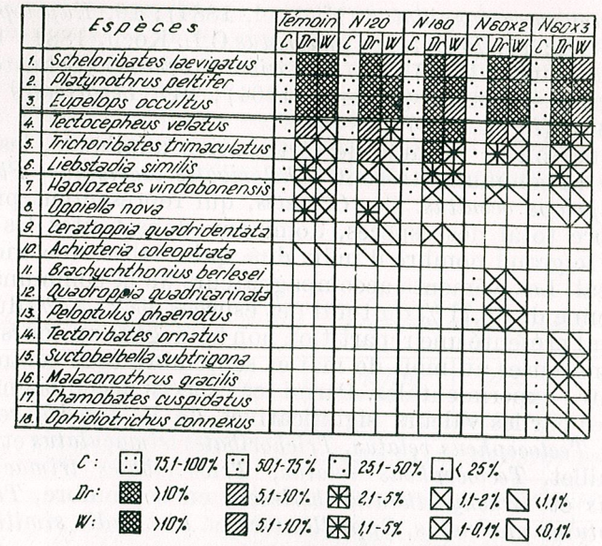


Fig. 1. — Mai, l'analyse cénologique de la communauté d'*Oribates*.

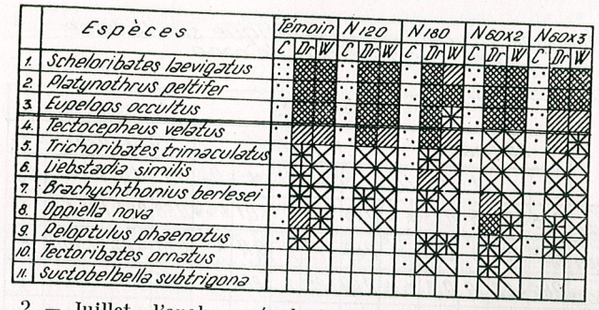


Fig. 2. — Juillet, l'analyse cénologique de la communauté d'*Oribates*.

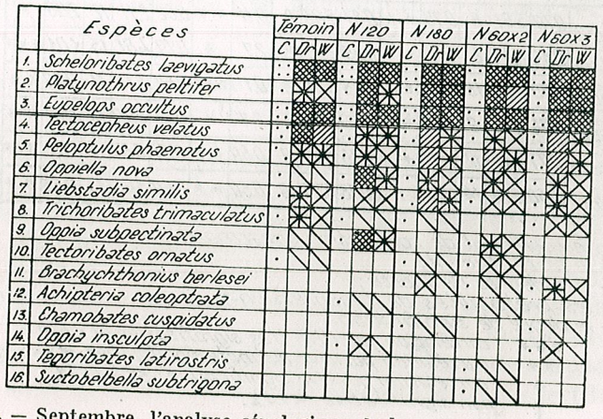


Fig. 3. — Septembre, l'analyse cénologique de la communauté d'*Oribates*.

1836); 15. *Chamobates cuspidatus* (Michael, 1884); 16. *Eupelops occultus* (C.L. Koch, 1836); 17. *Peloptulus phaenotus* C.L. Koch, 1884; 18. *Achipteria coleoptrata* (Linné, 1758); 19. *Tectoribates ornatus* (Schuster, 1958); 20. *Ophidiotrichus connexus* (Berlese, 1904); 21. *Tegoribates latirostris* (C.L. Koch, 1844) (fig. 1, 2, 3) [1], [8].

Quelle que soit la formule de fertilisation appliquée, les éléments édificateurs de la communauté sont *Scheloribates laevigatus*, *Platynothrus peltifer*, et *Eupelops occultus*. Ces *Oribates*, qui représentent presque 14, 29% du nombre total des espèces, dominent dans toutes les variantes analysées, par le grand nombre d'individus qui réalise une moyenne de 78,18% du total. Les éléments accompagnateurs de la communauté réalisent une moyenne de 85,71% du total des espèces et 21,82% du total des individus. Il sont présenté une répartition non homogène au cours du temps, étant plus nombreuses au mois de mai et septembre tout comme dans le cas des variantes expérimentales. Parmi les espèces accompagnatrices, on peut distinguer par les valeurs significatives de la densité relative, au mois de mai, *Tectocepheus velatus*, *Trichoribates trimaculatus* et *Liebstadia similis*; en juillet, *Tectocepheus velatus*, *Trichoribates trimaculatus*, *Liebstadia similis* et *Brachychthonius berlesei*; en septembre, *Tectocepheus velatus*, *Peloptulus phaenotus*, *Oppiella nova* et *Liebstadia similis*.

TABLEAU 2

Analyse de l'affinité cénotique sur la base des corrélations de rang

| | 1979 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 mai | | | | | 13 juillet | | | | | 5 septembre | | | | |
| | $r_s$ | $t$ | $l$ | $\alpha$ | $D_s$ | $r_s$ | $t$ | $l$ | $\alpha$ | $D_s$ | $r_s$ | $t$ | $l$ | $\alpha$ | $D_s$ |
| Témoin — $N_{120}$ | 0,88 | 4,32 | 8 | 0,23 | ** | 0,95 | 7,94 | 7 | <0,1 | *** | 0,58 | 2,27 | 10 | 5,3 | † |
| Témoin — $N_{180}$ | 0,70 | 2,27 | 8 | 2,6 | * | 0,88 | 5,17 | 8 | 0,11 | ** | 0,78 | 4,04 | 10 | 4,00 | * |
| Témoin — $N_{60}x_2$ | 0,80 | 4,22 | 10 | 0,18 | ** | 0,40 | 1,23 | 8 | 26 | † | 0,45 | 1,73 | 12 | 11,4 | † |
| Témoin — $N_{60}x_3$ | 0,77 | 3,98 | 11 | 0,18 | ** | 0,87 | 5,03 | 8 | 0,11 | ** | 0,70 | 3,10 | 10 | 1,12 | * |
| $N_{120}$ — $N_{180}$ | 0,79 | 3,61 | 8 | 0,70 | ** | 0,87 | 5,03 | 8 | 0,11 | ** | 0,62 | 2,71 | 12 | 2,00 | * |
| $N_{120}$ — $N_{60}x_2$ | 0,84 | 4,43 | 8 | 0,22 | ** | 0,69 | 2,72 | 8 | 2,7 | * | 0,79 | 4,61 | 13 | <0,1 | *** |
| $N_{120}$ — $N_{60}x_3$ | 0,70 | 3,53 | 13 | 0,38 | ** | 0,88 | 5,17 | 8 | 0,1 | *** | 0,67 | 3,15 | 11 | 0,80 | ** |
| $N_{180}$ — $N_{60}x_2$ | 0,74 | 3,32 | 9 | 0,93 | ** | 0,30 | 2,68 | 8 | 2,7 | * | 0,74 | 3,99 | 13 | 0,18 | ** |
| $N_{180}$ — $N_{60}x_3$ | 0,77 | 4,16 | 12 | 0,13 | ** | 0,76 | 3,53 | 9 | 0,69 | ** | 0,91 | 6,63 | 9 | <0,1 | *** |
| $N_{60}x_2$ — $N_{60}x_3$ | 0,50 | 2,16 | 14 | 5,4 | † | 0,78 | 3,75 | 9 | 0,50 | ** | 0,69 | 3,46 | 13 | 0,53 | ** |

$r_s$ – le coefficient de corrélation Spearman;
$t$ – le testé Student;
$l$ – n-2 degrés de liberté;
$\alpha$ – les probabilités de transgression-%;
$D_s$ – le degré de signification:
*** corrélation positive très significative: $\alpha < 0,1\%$;
** corrélation positive distincte significative: $1\% > \alpha > 0,1\%$;
* corrélation positive significative: $5\% > \alpha > 1\%$;
† non significative: $\alpha > 5\%$.



L'analyse de la corrélation entre le témoin et les variantes fertilisées et entre les variantes — en employant une méthode non paramétrique — (tabl. 2) a mis en évidence des valeurs positives. Entre le témoin et les variantes fértilisées on observe, au moins de mai et juillet, la prédominance d'une liaison positive distincte significative et au mois de septembre une diminution accentuée du degré de signification. Le degré de signification de la corrélation entre les variantes fertilisées ne présente pas de grandes différences.

TABLEAU 3

Les valeurs des diversités spécifiques et de l'équitabilité en fonction des doses d'engrais appliquées

| Variantes | S | | | N | | | H(S) | | | H(S)mx | | | Hr% | | | E% | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | Ju | S | M | Ju | S | M | Ju | S | M | Ju | S | M | Ju | S | M | Ju | S |
| Témoin | 9 | 9 | 10 | 129 | 92 | 145 | 2,314 | 2,705 | 2,240 | 3,169 | 3,169 | 3,321 | 73 | 85 | 67 | 77 | 100 | 60 |
| $N_{120}$ | 9 | 8 | 11 | 134 | 64 | 127 | 2134 | 2,362 | 2,150 | 3,169 | 2,939 | 3,459 | 67 | 78 | 62 | 66 | 87 | 54 |
| $N_{180}$ | 8 | 9 | 11 | 68 | 92 | 143 | 2,685 | 2,643 | 2,337 | 2,999 | 3,169 | 3,459 | 89 | 83 | 67 | 112 | 72 | 63 |
| $N_{60}x_2$ | 9 | 10 | 13 | 87 | 60 | 134 | 2,206 | 2,713 | 2,614 | 3,169 | 3,321 | 3,700 | 69 | 81 | 70 | 66 | 90 | 61 |
| $N_{60}x_3$ | 13 | 9 | 11 | 119 | 71 | 146 | 2,387 | 2,328 | 2,618 | 3,700 | 3,169 | 3,459 | 64 | 70 | 78 | 53 | 77 | 72 |

S – le nombre total des espèces; N – le nombre total d'individus
H(S) – la diversité spécifique H(S)mx – la diversité maximale ou hypothétique
Hr% – la diversité relative E% – l'équitabilité
M – 25 Mai 1979
Ju – 13 Juillet 1979
S – 5 Septembre 1979

La diversité spécifique des populations d'*Oribates* (tabl. 3) a présenté, en général, des valeurs proches de celle du témoin. Les fluctuations dans le temps de la diversité ont une tendance pareille à celle du témoin, dans toutes les variantes fertilisées à l'exception de la variante $N_{180}$. Dans le cas du témoin on a remarqué une augmentation de la diversité réelle au mois de juillet, lorsque les populations d'*Oribates*, malgré leurs effectifs numériques inférieurs, se sont caractérisées par de densités relatives plus proches. Au mois de septembre on enregistre une diminution accompagnée d'une tendance d'augmentation de la diversité maximale due à l'augmentation du nombre des espèces. En échange, la variante $N_{180}$ où l'on applique au printemps intégralement les doses les plus élevées d'engrais minéraux diminue au mois de mai, en ce qui concerne le nombre d'espèces et d'individus, mais on peut remarquer une répartition plus équilibrée des espèces. C'est un phénomène qui se reflète dans les valeurs accrues de la diversité réelle et dans les valeurs diminuées de la diversité maximale. En juillet, les valeurs de la diversité réelle se maintiennent à peu près les mêmes, tandis que les valeurs de la diversité maximale deviennent égales à celles du témoin, à cause du nombre égal des espèces. En septembre, la valeur de la diversité réelle de cette variante fertilisée diminue

et s'approche de celle du témoin. La diversité maximale présente une tendance d'augmentation.

L'équitabilité est caractérisée, en général, par de grandes valeurs, illustrant le nombre réduit d'espèces qui vivent dans ce biotope dont les effectifs de population tendent vers une répartition équiproportionnelle. Tandis que les valeurs de l'équitabilité du témoin et les variantes $N_{120}$, $N_{60\times2}$, $N_{60\times3}$ se modifient dans le même sens atteignant les plus grandes valeurs en juillet, dans la variante $N_{180}$ la valeur maximale s'enregistre en mai.

## DISCUSSIONS

La physionomie de la communauté d'*Oribates* reste le même quelle que soit la formule de fertilisation. Dans toutes les situations analysées il y a trois espèces *(Scheloribates laevigatus*, *Platynothrus peltifer* et *Eupelops occultus)* à large valence écologique qui dominent par leur productivité numérique. Les autres espèces qui forment la communauté sont accessoires ou accidentelles. Parmi celles-ci il y a, quand même, certaines qui présentent des populations relativement grandes et que nous considérons significatives pour l'évolution ultérieure de la communauté. Au cours des années antérieures, sur un terrain fertilisé depuis longtemps, *Liebstadiasimilis* et *Tectocepheus velatus* ont eu les caractéristiques des édificateurs. La première année de fertilisation ne modifie pas la position des espèces par rapport à leur domination. Au cours du temps d'une variante à l'autre, on a saisi certaines fluctuations des effectifs des populations d'*Oribates* qui mènent à l'augmentation ou à la diminution des différences entre leurs densités relatives. C'est ce qui finalement détermine les petites variations de la stabilité des communautés analysées.

Dans les cas du témoin, ces fluctuations sont dues à l'influence de l'ensemble des facteurs climatiques et bioédafiques.

Dans les variantes fertilisées nous avons remarqué deux situations différentes. Il y a d'un part les variantes $N_{120}$, $N_{60\times2}$, $N_{60\times3}$ où il semble que les fluctuations de la stabilité sont influencées surtout par l'ensemble de facteurs climatiques et bioédafiques et d'autre part la variante $N_{180}$ où l'on ressent plus évidemment l'effet de la fertilisation.

## CONCLUSIONS

Les recherches entreprises ont mis en évidence que l'effet de la première année de fertilisation avec des engrais minéraux basés sur P, K et N n'apparaît pas sur la structure de la communauté d'*Oribates* que grâce à de petites fluctuations de la stabilité cénotique décelées dans le cas de l'administration intégrale des doses $P_{83}$, $K_{108}$ et $N_{180}$.

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*Iaşi, Calea 23 August 11*

PROFESSEUR GHEORGHE LUPAȘCU

(1908—1979)

Un an seulement apres avoir été fêté sous les auspices de notre Académie pour l'anniversaire de ses soixante-dix ans, le professeur Gheorghe Lupaşcu s'est soudainement éteint le 29 novembre 1979 à Bucarest, à la consternation de ses collaborateurs et amis. Sa fin est une lourde perte pour la parasitologie roumaine, dont il était devenu la personnalité centrale et qu'il représentait avec autant d'autorité à l'étranger. Il nous a semblé donc un devoir de retracer brièvement ici ses réalisations professionnelles.

Né à Botoşani, le 22 mars 1908, il fait ses études au lycée de la même ville du nord de la Moldavie. Gh. Lupaşcu sera ensuite successivement promu licencié de la Faculté des Sciences (1932) et docteur ès sciences naturelles de l'Université de Bucarest (1945). Encore étudiant, il travaille auprès du prof. Gheorghe Zotta tant au Laboratoire de Zoologie médicale et Parasitologie à la Faculté de Medecine de Bucarest, que dans la Section de parasitologie de l'Institut de sérums et vaccins du prof. Jean Cantacuzène.

Il poursuit une spécialisation en Italie, grâce à une bourse de l'Académie Roumaine, effectuant des recherches d'entomologie médicale à l'« Istituto Superiore di Sanita » de Rome (1940) où il est apprécié par le prof. A. Missiroli et A. Corradetti. Appelé maître de conférences et professeur de parasitologie (1947) à l'Institut de Médecine de Timişoara, il y devint également recteur.

Il est élu membre correspondant de l'Académie de la République Populaire Roumaine (1948) et participe auprès du prof. M. Ciucă à l'organisation des campagnes antipaludéennes et à l'éducation d'un personnel spécialisé dans ce but. Transféré comme professeur à la chaire de parasitologie de la Faculté de Médecine de Bucarest (1952), il assume également la direction de l'Institut « I. Cantacuzino » et va demeurer chef de la Section de parasitologie du même institut (1956).

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En sa qualité d'expert de l'Organisation Mondiale de la Santé (W.H.O.) pour la malaria (1956), il va utiliser son expérience dans l'éradication de ce fléau en Roumanie pour combattre cette pandémie dans les pays africains. Outre la mise en évidence de la phase exoérythrocytaire dans le cycle du *Plasmodium malariae* (1966), ses travaux portent sur des problèmes du plus haut intérêt parasitologique, tels l'hydatidose (1968), la trichinellose (1970), la trichomonase uro-génitale, etc., qui firent l'objet de livres publiés dans les éditions de notre Académie.

Depuis 1960, en tant que président de la Section de parasitologie de l'Union des Sociétés scientifiques médicales de Roumanie, il a dirigé une série d'importantes réunions avec participation internationale. Membre titulaire de l'Académie des sciences médicales de Roumanie (1969), il fut aussi coopté dans les principales sociétés de parasitologie de l'étranger (Angleterre, France, Etats-Unis, etc.) En 1979, au Congrès parasitologique de Varsovie, il a reçu cette marque d'estime d'être élu dans le comité exécutif de la « World Federation of Parasitologists ».

Nous renouvelons un hommage ému à la mémoire du professeur Gheorghe Lupaşcu qui a su efficacement appliquer sa compétence de biologiste à la lutte mondiale contre la menace parasitaire des collectivités humaines. Tous ceux qui l'ont connu ne pourront pas séparer ses mérites scientifiques de l'affabilité de son accueil et de ses prédilections artistiques.

*Radu Codreanu*



## CENTENAIRE DE LA NAISSANCE DU SAVANT BIOSPÉOLOGISTE RENÉ JEANNEL

La Section des Sciences biologiques de l'Académie de la République Socialiste de Roumanie en commun avec l'Institut de Spéologie « Emile Racovitza » et l'Institut Central de Biologie ont organisé l'anniversaire du centenaire de la naissance du savant français René Jeannel dans la salle de festivités de l'Académie, le 18 octobre 1979.

La séance, honorée par la présence du prof. Şerban Ţiţeica, viceprésident de l'Académie, prof. Maurice Fontaine, membre de l'Académie des Sciences de Paris, M.M. André Michel et Alain Warzée, conseillers culturels près l'Ambassade de France, a été ouverte par l'académicien Radu Codreanu qui a souligné l'ampleur de l'œuvre biospéologique de René Jeannel, son étroite collaboration avec Emile Racovitza, les services qu'il a rendus à notre pays et sa qualité de membre de notre Académie.

Ont suivi le prof. Traian Orghidan, directeur de l'Institut de Spéologie, qui a retracé la vie et la personnalité de René Jeannel et prof. Maurice Fontaine, ancien directeur du Muséum National d'Histoire Naturelle à Paris, qui a évoqué la féconde activité de René Jeannel comme professeur au Muséum, après son départ de Roumanie. Ensuite les chercheurs de l'Institut de Spéologie, à savoir dr. Gheorghe Racovitza (Cluj-Napoca) a exposé la magnifique œuvre entomologique de René Jeannel et dr. Ionel Tabacaru (Bucarest) a mis en évidence sa contribution éminente à la reconstitution de l'histoire paléogéographique des faunes continentales. Finalement, Valeriu Puşcariu, ancien collaborateur de René Jeannel pendant son séjour en Roumanie, a rappelé ses souvenirs sur l'activité déployée par le savant français à l'Université de Cluj-Napoca.

En même temps, M. Claude Delamare-Deboutteville, professeur d'Ecologie au Muséum à Paris, en tant que disciple et biographe de René Jeannel, a bien voulu envoyer un texte sur la portée générale des recherches de l'illustre savant. Ce fut une manifestation attestant la vigueur de la coopération scientifique franco-roumaine et destinée à rendre un juste hommage à la mémoire de René Jeannel, dont l'œuvre massive a rénové la systématique évolutive à l'appui de la théorie mobiliste d'Alfred Wegener sur la dérive des continents.

*Radu Codreanu*

## CENTENAIRE DE LA NAISSANCE DU SAVANT ZOOLOGISTE IOAN BORCEA

Inscrit dans le programme de l'UNESCO, l'anniversaire du centenaire de la naissance du professeur Ioan Borcea s'est déroulé sous les auspices de la Section des Sciences biologiques de l'Académie de la République Socialiste de Roumanie, en collaboration avec l'Institut Central de Biologie, dans la salle de festivités de l'Académie, le 28 novembre 1979.

En présence du prof. Şerban Ţiţeica, vice-président de l'Académie, la série des communications commémoratives a débuté par l'allocution de l'académicien Radu Codreanu qui a relevé la persistance du souvenir de la personnalité puissante et de l'œuvre scientifique féconde de Ioan Borcea qui a porté sur deux problèmes majeurs : l'introduction du principe de la lutte biologique contre les insectes nuisibles à l'agriculture et la connaissance de la faune et de l'écologie de la Mer Noire et de ses étangs littoraux dans le but d'une meilleure productivité.

A son tour, Olga Necrasov, professeur à l'Université de Jassy et membre correspondant de l'Académie, a retracé la vie de Ioan Borcea, ses mérites comme chef d'école et à promouvoir la culture scientifique en Roumanie, son prestige comme représentant de la zoologie roumaine à l'étranger. Vasile Gh. Radu, professeur à l'Université de Cluj-Napoca et membre correspondant de l'Académie, a montré l'intérêt fondamental pour la morphologie comparée des vertébrés de la thèse de doctorat de Ioan Borcea sur la structure et l'ontogénie du rein des sélaciens, soutenue sous la présidence d'Yves Delage à la Sorbonne (Paris, 1905) et publiée dans les Archives de Zoologie expérimentale et générale. Le prof. Mihai Constantineanu et dr.

Raoul Constantineanu de l'Université de Jassy, ont montré la contribution importante de Ioan Borcea à l'entomologie générale et appliquée et la voie fructueuse qu'il a ainsi ouverte à de nombreux continuateurs.

Dr. Mihai Băcescu, membre correspondant de l'Académie, disciple de Ioan Borcea, a relié les recherches de celui-ci sur les ressources biologiques du littoral roumain de la Mer Noire au développement actuel des explorations océanologiques et à leur capacité de satisfaire les besoins de l'expansion humaine. L'académicien Petru Jitariu a attesté le rôle de la Station marine que Ioan Borcea avait fondée à Agigea (1926) d'avoir fait éclore, parallèlement aux études zoologiques, une active école roumaine de physiologie écologique.

La figure de Ioan Borcea acquiert ainsi l'éclat d'une permanence de la biologie roumaine par la solidité de ses liens avec les réalités nationales et l'actualité scientifique mondiale.

*Radu Codreanu*

## CENTENAIRE DE LA NAISSANCE DU SAVANT HISTOLOGISTE ION A. SCRIBAN

C'est sous l'égide de la Section des Sciences biologiques de l'Académie de la République Socialiste de Roumanie avec la participation de l'Institut central de Biologie que fut célébré l'anniversaire du centenaire du professeur I.A. Scriban dans la salle de fêtes de l'Académie, le 12 décembre 1979.

Dans son allocution d'ouverture, l'académicien Radu Codreanu a rappelé les principales étapes de la carrière scientifique de I.A. Scriban, d'abord à l'Université de Jassy, où il a été l'émule et le collaborateur du prof. Paul Bujor et ensuite à l'Université de Cluj-Napoca, où en tant que titulaire de la chaire de zoologie et d'anatomie comparée, il a fondé une importante école d'histologie et d'histophysiologie et a acquis une notoriété internationale dans l'étude morphologique des hirudinées.

A tour de rôle, prof. Crustalo Acrivo-Miclea de la Faculté de médecine de Timişoara, ancienne assistante du professeur I.A. Scriban a évoqué la vie et la personnalité du savant histologiste ; prof. Vasile Gh. Radu, membre correspondant de l'Académie et son successeur à la chaire de l'Université de Cluj-Napoca, a mis en relief les aspects les plus significatifs de son œuvre scientifique, qui a également porté sur l'évolution structurale de la branchie des poissons et sur les myopathies primitives chez l'homme ; en dernier lieu, prof. Emil Roşu de l'Institut Agronomique de Jassy et autrefois son assistant, a retracé l'activité universitaire du professeur I.A. Scriban et les services qu'il a rendus à la propagation de la culture roumaine en Transylvanie.

Malgré sa perte prématurée, au même âge que son ancien collègue à Jassy, Ioan Borcea, le professeur I.A. Scriban demeure par son attachement à la recherche microscopique, l'un des maîtres de la cytologie roumaine, dont l'apport est constamment présent dans la littérature zoologique internationale.

*Radu Codreanu*



N. MANOLESCU, V. CIOCNITU, C. DIMITRIU, *Ultrastructura unor celule sanguine în microscopia electronică de baleiaj* (L'ultrastructure de certaines cellules sanguines en microscopie électronique à balayage), Ed. ştiinţifică şi enciclopedică, Bucarest, 1979, 151 p. 107 fig.

Les auteurs appartenant à l'Institut « Pasteur » de recherches vétérinaires et à l'Institut des sciences biologiques de Bucarest, apportent dans ce volume leurs résultats de trois ans d'investigations en microscopie électronique à balayage de 280 cas d'hémopathies des principaux animaux domestiques et de l'homme. Leur but est de montrer que cette technique d'application récente permet de mettre en évidence des caractères différentiels très nets de la cytoarchitecture superficielle dans les lignées lympho-plasmocytaire et monocyto-histiocytaire, à l'état normal et pathologique.

L'ouvrage est divisé en 4 chapitres, dont le premier représente une introduction technique aussi bien générale dans l'utilisation du microscope électronique à balayage („scanning") que en ce qui concerne les conditions nécessaires à la realisation d'images d'une haute qualité. Le chapitre II expose les méthodes de prélèvement des préparations biologiques, selon qu'elles proviennent du sang ou de différents organes hématopoïétiques et il s'y ajoute toute la série d'opérations techniques de la préparation des cellules à étudier. Le chapitre III renferme la description comparée de la diversité constatée en microscopie électronique à balayage des cellules sanguines normales en rapport avec les stades de leur genèse à partir du thymus ou de la moelle osseuse, la lignée histio-monocytaire étant également envisagée. Le chapitre IV et dernier, le plus substantiel du volume, traite des aspects de pathologie sanguine décelés au balayage, notamment de la transformation sarcomateuse des éléments proliférants d'après leur cytoarchitecture de surface offrant des caractéristiques différentielles dans les divers types de leucémies, identifiées chez l'homme, les mammifères, et les oiseaux domestiques.

En conclusions, l'étude tridimensionnelle des cellules sanguines en balayage, outre un intérêt théorique, est susceptible de fournir de nouvelles possibilités d'un cytodiagnostic assez économique des hémopathies malignes. C'est pourquoi, ce livre richement illustré et pourvu de références bibliographiques, jouira certainement d'un accueil favorable auprès de différents chercheurs en biologie, zootechnie, médecine vétérinaire et humaine.

*Radu Codreanu*

MANEA I. GHEORGHE, *Sturionii. Biologie. Sturionicultură şi amenajări sturionicole* (Les esturgeons. Systématique. Biologie. Élevage et aménagement), I^er^ vol., 244 p., 59 fig., 48 tabl., Ed. Ceres, Bucureşti, 1980

Ce livre constitue la première monographie mondiale sur les Esturgeons, surtout sur ceux des contrées européennes.

Expert dans la pisciculture en général et en sturioniculture en spécial, l'auteur travaille dans ce domaine depuis de nombreuses années. Grâce à ses travaux, on a obtenu des résultats positifs et de grande valeur économique dans les élevages des esturgeons dans le Delta du Danube.

Le livre, fruit de son expérience, est basé sur plus de 240 titres bibliographiques, dont 32 % appartenant à des auteurs roumains.

Le livre est divisé en deux grandes parties :

Dans la première partie on expose la systématique et la biologie des Esturgeons dans leur milieu naturel, l'origine de ces Ganoïdes et on décrit amplement les presque 20 espèces d'Acipenserides, les représentants des eaux qui se déversent dans la mer Noire étant décrits en détail ; on présente ensuite des données sur la valeur alimentaire et sur la production mondiale et régionale de ces poissons.

La deuxième partie contient les résultats obtenus en ce qui concerne la reproduction, l'élevage et les aménagements spécifiques nécessaires à leur croissance, à leur nutrition, à leur maturation sexuelle et de nouveau à leur reproduction. Parallèlement, on décrit les modalités d'obtenir une nourriture adéquate pour les larves, les alévins et les adultes, c'est-à-dire comment peut-on produire des puces d'eau, des vers olygochètes, des Artemia et des larves de Chironomides. On s'occupe des moyens d'acclimatation des esturgeons dans de nouvelles conditions naturelles ; enfin, on présente plusieurs systèmes de constructions et installations spéciales pour la sturioniculture, surtout ceux qui ont donné de bons résultats dans le Delta du Danube.

Dans la dernière partie, l'auteur présente les perspectives de la sturioniculture en Roumanie et dans le monde entier, en se basant sur les conclusions qui résultent de ses travaux, obtenus pendant une vie entière dédiée à l'étude des poissons.

Le livre se termine par un résumé dans les langues anglaise, française et russe.

Je pense que le livre de Gh. I. Manea, première mondiale dans le domaine des Esturgeons, peut constituer la base de discussion d'une rencontre internationale sur le problème et l'avenir des esturgeons, classe de poissons de grande valeur alimentaire et gastronomique, mais fort sensibles à la pollution aquatique, qui menace déjà plusieurs de leurs espèces de disparition.

Le livre concerne de près les biologistes, les pisciculteurs, les médecins et tous ceux qu s'intéressent à la vie très intéressante des Esturgeons.

*Eugen A. Pora*

REVUE ROUMAINE DE
BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

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## AVIS AUX AUTEURS

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

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

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

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

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

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