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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

TOME 27 Nº 1

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SOMMAIRE

ACADEMICIAN EUGEN A. PORA

On the 28th of October 1981, Academician Eugen A. Pora passed away in full power of creation. He dedicated more than fifty years of his life to research work in animal physiology and his thirst for knowledge was quenched only by death.

Born in 1909 at Bunești, Brașov county, E. A. Pora attended college and university courses in Cluj, graduating in 1932 with a paper on "The Anatomy and Physiology of the Tegument in Animals". In 1938, he submitted his doctoral disertation (directed by Prof. Dr. Ar. Grădinescu) "The Influence of Continuous Electric Current on the Branchial Permeability of Fish". Both works won him a "magna cum laude" notation.

Between 1934—1936, he undertook a series of specialization studies in France: at the Sorbonne General Physiology Department (with Prof. P. Portier), at the Oceanographic Institute in Paris (with Prof. M. Fontaine) and the Marine Biological Station in Roscoff (with Profs Perez and G. Teissier). In 1958, he spent a two-and-a-half-month study in Moscow at the All-Union Institute of Veterinary Research, Domestic Animals Physiology Department (Prof. A. Kudriavtsev).

In 1944, he was appointed professor at the Animal Physiology Department of the University of Iași and from 1946 to 1972, when he retired, Pora discharged the same function at the University of Cluj.

A passionate researcher and a good organizer, he equipped his laboratory with modern installations, fact that placed the training of his pupils on a solid foundation.

E. A. Pora was a corresponding member of the Romanian Academy from 1939 to 1963 and from that time on he had the status of full member.

REV. ROUM. BIOL. — BIOL. ANIM., TOME 27, Nº 1, P. 3–4, BUCAREST, 1982

He was also member of a series of foreign bodies : the International Association of Limnology (1956), the Physiologist Association (1957), the New York Academy of Sciences, the European Society of Endocrinology (1967), the European Society of Radiobiology, vice-president and then president (1970) of the Mediterranean Association for Marine Biology and Oceanography, doctor "honoris causa" of the University of Lyon, France.

Alone or in cooperation, E. A. Pora published over 456 works on the physiology and ecophysiology of animals, 15 teaching manuals, over 20 monographs, very appreciated by Romanian and foreign specialists, as well as some 450 biology articles ; he held numerous radio and tv conferences (177 between 1972—1977) and mentained relations with more than 220 researchers, teachers and professors from Romania and abroad.

Since 1957, he acted as major professor to the doctoral theses of over 60 researchers.

From his vast activity we would recall a few fields of interest : the study of animal membrane permeability to saults under the influence of the continuous electric current ; the chemical differences between sexes in nonvertebrates and vertebrates, considered to be analogous to the secondary morphological characters. For many years, he studied oceanographic problems, the effects of the variations of certain environmental factors on the marine organisms. In 1962, he continued his researches in the Indian Ocean on the "Viteazul" ship. Of major importance were his studies on the adaptation of aquatic animals to different marine salinity levels (103 papers) carried out at the Agigea Research Station (Constanța). These studies finally led him to the concept of "rhopy" and "homeorhopy", accepted also by the foreign specialized literature. He founded a school of cophysiology. Pora's concerns centered also on the study of the physiology of the ontogenetic development of the digestive, circulatory, breathing, metabolic and endocrine functions (137 works), on problems of animal breeding and fish rearing, with practical applications in production.

By crossing Sussex and Rhode-Island hen breeds, he obtained a new type of hen called "Lerminata of Bontide".

As a result of the researches carried out on the multi-chambered stomach symbients of prime breed wool sheep he created an original way of feeding lambs, which stimulated their growth.

In 1964, his long and meritorious didactic activity won him the title of Emeritus Professor. E. A. Pora had spared no efforts in modernizing university and high school biological teaching.

P. Jitariu and Șt. Vancea

NOUVELLE CONTRIBUTION À L'ÉTUDE DES APSEUDOIDEA *LEVIAPSEUDES LONGISSIMUS* N. SP. DES EAUX SUD-OUEST AFRICAINES

PAR

MIHAI BĂCESCU

En 1977 la Campagne Walvis, organisée par le CNRS, a exploré la faune benthique profonde du Secteur SO de l'Afrique. Dans un échantillon de cette campagne envoyé par le Dr. Segonzac, j'ai trouvé un magnifique Apseudoidea, que je décris comme *Leviapseudes longissimus* n. sp.

Diagnose. Tanaidacé géant (♀ = 32 mm) avec 5 pléopodes minuscules, dont les deux rames sont fusiformes, uniarticulées et ayant seulement 2 (rarement 1) setae fines apicales. La maxillule au palpe uniarticulé. Les pléonites plus longs que larges.

Matériel (envoyé par CENTOB) [1]. Une femelle avec des embryons, mésurant 32 mm. St. KG 12 de la Campagne Walvis, 4600 m de fond, SO de l'Afrique ; 10°47′51″N ; 42°40′41″O), le 20.XI.1977.

Description de la femelle type. Le corps très long (9 fois plus long que la largeur maximale au niveau du premier péréionite libre) car tous ses segments, excepté les thoracomères 1 et 2, sont plus longs que larges (Fig. 1 A). Le tégument parfaitement glabre, calcifié mais non friable, blanc luisant. Vu que la femelle n'était pas loin de la saison de la mue, toutes les lignes de déhiscence étaient visibles.

La carapace plus longue que large, avec des plis puissants qui circonscrivent les chambres respiratrices et les apophyses postoculaires. Le rostre triangulaire, non incliné ventralement ; les bords de sa base sont droits. Les lobes oculaires larges, pointus, sans omatidies ; sur la face ventrale, ils présentent une cicatrice ronde, comme si un phanère s'en était détaché. Les lobes postoculaires larges, brusquement pointus, dirigés frontalement. Entre eux et les chambres respiratrices apparaît clairement une expansion demi-circulaire qui tient du plastron de la carapace, c'est-à-dire de sa partie courbée ventralement.

Les péréionites (Fig. 1 A et A_1) s'accroissent en longueur, les plus longs étant le V^{e} et le VI^{e}, alors que le plus court est le I^{er} qui est en même temps le plus large (presque deux fois plus large que long, tandis que le plus long (le V^{e}) est presque deux fois plus long que large, sans compter la plaque coxale du péréiopode II d'ailleurs très courte, comme un triangle équilatéral, faiblement pointue. De petites épines hyposphéniales pointues seulement entre les chélipèdes et les péréiopodes VII ; les autres sternites manquent de toute trace d'épine hyposphéniale la femelle étant

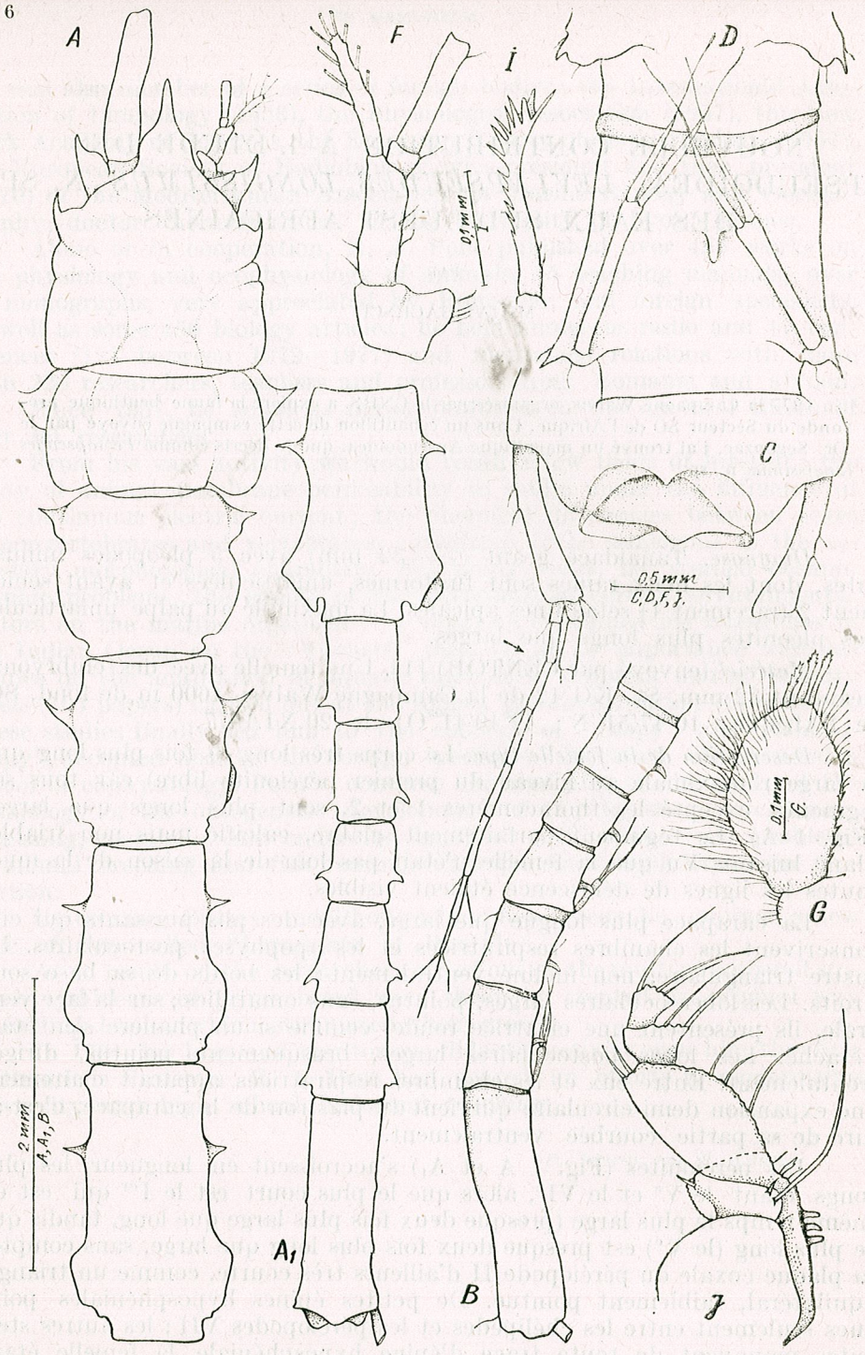

Fig. 1. — *Leviapseudes longissimus* ♀ ad. — A et A_1, femelle gravide, vue tergale ; B, son abdomen, vue latérale ; C, partie terminale du pléotelson et base des uropodes ; D, pléonite VI, vue ventrale ; F, antenne ; G, palpe droit du labium ; I, phanère palmé de la maxille ; J, maxillipède.

porteuse d'embryons. Le péréionite I (Fig. 1 A_1 et B) n'a pas d'épines latérales ; le II^e a deux expansions spiniformes antérolatérales ; les autres ont chacun une puissante apophyse spiniforme dans le tiers antérieur. Le pléon est formé de segments presque égaux, cylindriques (ils apparaissent rectangulaires en vue tergale), nettement plus longs que larges. Ils sont tous dépassés latéralement par une large apophyse postérieure qui finit par un prolongement spiniforme à direction caudale (Fig. 1 A_1).

Le pléotelson cylindrique tout aussi long que 2,5 pléonites pris ensemble ; son ouverture cloaquale comme dans la fig. 1 C, sans aucune soie. Tous les pléonites sont pourvus de courtes épines hyposphéniales courbées caudalement (Fig. 1 B et D).

Appendices. L'antennule (Fig. 2 E) avec l'article proximal de la base plus court que la carapace, presque lamellaire, puissamment aplati dorso-ventralement (il est 4 fois plus large que haut) ; son flagelle interne présente 6 articles ; celui externe en a 24.

L'antenne (Fig. 1 F) à l'article basal proximal avec un prolongement interne presque circulaire, avec 3—4 poils ; l'écaille avec 8—9 setae ; l'endopodite avec 8—10 articles.

L'épistome ressemble à une cuirasse bombée sur la ligne médiane et il est dépourvu d'épines. Le labrum est excavé, avec une petite proéminence au centre de l'excavation.

La mandibule et surtout son palpe, tout comme chez *Leviapseudes conspicuus* (Fig. 56, g-i Lang) ; pars molaris ovale, molle, avec rien qu'une ombre de chitinisation.

Le labium (Fig. 1 G) au palpe court, oval, avec beaucoup de cils notamment sur la marge externe, finissant par 3 phanères à l'apex serrate.

La *maxillule au palpe uniarticulé*, avec 7 setae, au bout pareil à un crochet d'une part et finement serrate de l'autre ; la seta terminale (distale) est presque deux fois plus longue que les autres (Fig. 2 H). La maxille a une épine palmée sur le corps médian (Fig. 1 I).

Le maxillipède (Fig. 1 J) commun au genre ; l'article basal aux marges droites ; son endite (Fig. 2 K) avec l'habituelle seta palmée dépassant le front des phanères bifides ; trois rétinacles couverts de minuscules épines-crochets.

Les chélipèdes et les péréiopodes II font défaut ; il n'y a que la plaque épimérale des derniers, largement triangulaire, qui est restée. Le reste des péréiopodes, pareils à celui de la fig. 2 L, ont de longues dactylogriffes, beaucoup plus longues que le propodus (Fig. 2 M, N), excepté le péréiopode V dont la dactylogriffe est plus courte que lui (Fig. 2 O). Le carpe de tous les péréiopodes est plus long que le méros. Le péréiopode VII (Fig. 2M) présente un petit peigne courbé avec des lamines très courtes.

Les pléopodes réduits ; ils ne dépassent pas la longueur d'un pléonite et ont une structure aberrante ; leur base est longue et nue. Ils ont deux branches pointues, plus courtes, chacune pourvue d'un poil long, nu (Fig. 1 D) et respectivement 2 (à l'endopodite) (Fig. 2 R).

Les uropodes (Fig. 1 C) à insertion sous-terminale ; la moitié de leur base dépasse le bout du pléotelson et a seulement 3 poils ; leur exopodite est formé de 7 articles et leur endopodite de 27 + 28.

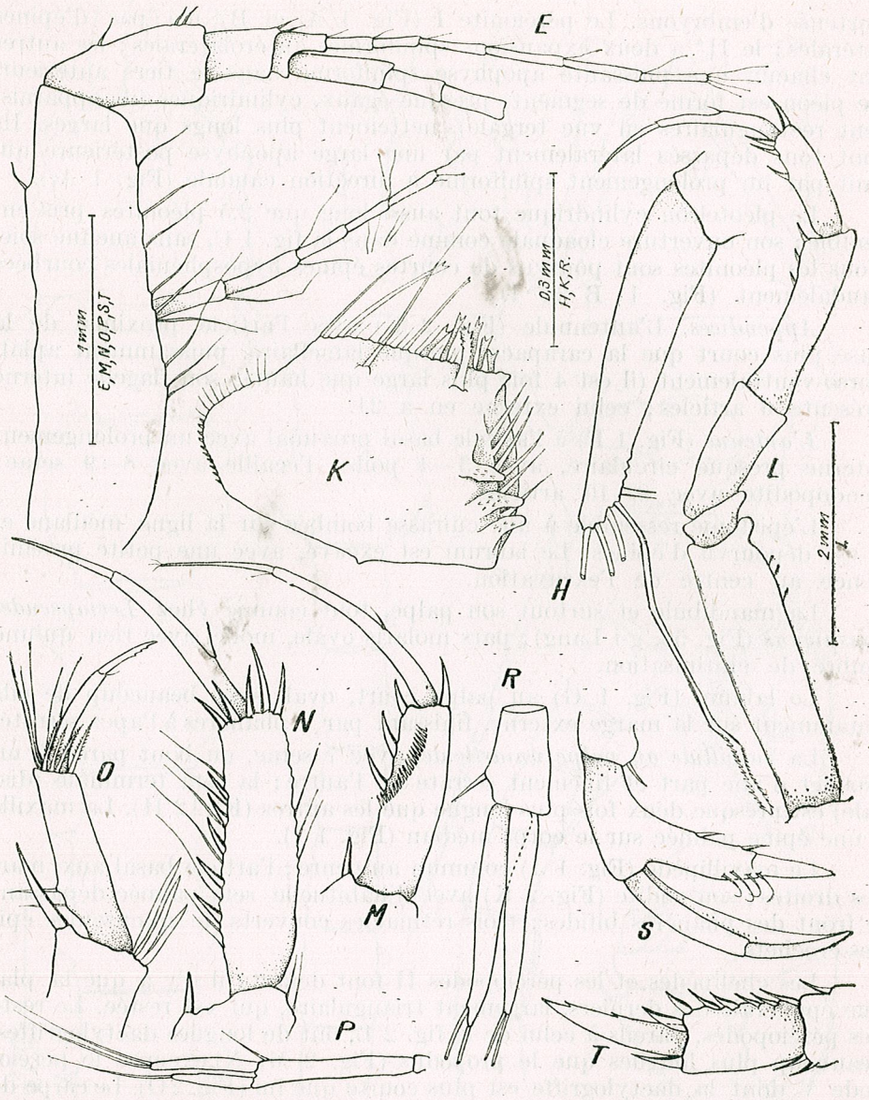

Fig. 2. — *Leviapseudes longissimus*. E, antennule ; H, palpe uniarticulé de la maxillule ; K, endite du maxillipède ; L, péréiopode VII M, sa partie distale ; N, O, idem, des péréiopodes III et V ; P, pléopode I ; R, pléopode V ; S, chéla d'un embryon prêt d'être libéré du marsupium ; T, dactyle et propodus du péréiopode II du même embryon.

La femelle portait 33 embryons prêts d'être évacués. Le péréiopode I avec un puissant exopodite, l'aspect de la chéla (en train de muer), comme chez l'embryon de la fig. 2, S. Le péréiopode II à 7 épines sur la marge interne et ventrale (Fig. 2 T).

L'holotype, ♀ adulte, déposé dans le Laboratoire de Crustacés du Muséum d'histoire naturelle de Paris.

Observations. Leviapseudes longissimus avec ses 32 mm est le tanaidacé le plus long connu jusqu'aujourd'hui après *Gigantapseudes adactylus* Kudinova-Past [2], celui-là ayant 25 à 37 mm. Les caractéristiques principales de cette espèce — du moins de la femelle connue — sont les suivantes : le palpe non articulé de la maxillule et la morphologie des pléopodes ; ces derniers, tous pareils, sont en général petits, ne dépassant pas la longueur d'un pléonite, avec deux branches presque fusiformes ; tant l'exopodite, qui est un peu plus court et non articulé que l'endopodite finissent par une ou deux setae, fines, plus longues que les branches-mêmes (Fig. 1 D) ; seul le pléopode V présente à l'exopodite aussi 2 setae, l'une des setae de l'endopodite étant fixée sous-terminalement non pas apicalement (Fig. 2 R). Par cette bizarre réduction, notre espèce rappelle d'une certaine manière seulement *Apseudes aberrans* Lang [3].

Par sa forme extérieure, par l'aspect des segments et par la morphologie des pléonites, *L. longissimus* ne s'approche d'aucune des 11 espèces connues du genre.

Reçu le 23 mars 1981

Musée d'histoire naturelle « Gr. Antipa »
Bucarest, Kiseleff 1

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DEUX ESPÈCES NOUVELLES DES GENRES *CHEILOSIA* MEIGEN ET *MERODON* MEIGEN (DIPTERA, SYRPHIDAE)

PAR

VL. BRĂDESCU

In the present work two news species, i. e. *Cheilosia herculana* sp. n. and *Merodon dobrogensis* sp. n. are described. The author made a comparative study of the species *Cheilosia armeniaca* Stackelberg. 1960, and *Merodon flaviventris* Sack, 1932, with which these new species have resembling characters.

Cheilosia herculana sp. n.

L'espèce appartient au groupe A conformément à la classification de Sack. Moyennant cette clef, nous arrivons au complexe des espèces représenté par *Cheilosia armeniaca* Stackelberg, *Ch. caerulescens* Meigen et *Ch. kerteszi* Szilady.

Notre espèce ayant des ressemblances évidentes avec *Cheilosia armeniaca* Stackelberg, nous présentons un tableau comparatif pour souligner les différences spécifiques aux mâles, la femelle de *Ch. armeniaca* Stackelberg étant inconnue :

Cheilosia herculana sp. n.	*Cheilosia armeniaca* Stackelberg
♂	♂
Corps noir — olivâtre.	Corps noir.
Front à pilosité blanchâtre ; á la partie postérieure quelques poils noirs.	Front à pilosité blanche.
Triangle ocellaire à pilosité noire.	Triangle ocellaire à pilosité blanche.
Antennes bruns-café.	Antennes noires.
Arista pubescente.	Arista nue.
Mésonotum à ponctuation fine.	Mésonotum à ponctuation grossière.
Ailes : t-m placée en angle aigu sur r_{4+5}.	Ailes : t-m placée en angle droit sur r_{4+5}.
Abdomen oval, surpasse la longueur de thorax + tête.	Abdomen oval, ne surpasse pas la longueur de thorax + tête.
Longueur du corps : 10—11,5 mm.	Longueur du corps : 8,5 mm.

MÂLE

Tête. Yeux nus. Front noir-olivâtre, à ponctuation grossière, évidemment sillonné, à pilosité blanchâtre épaisse ; en arrière quelques poils noirs. L'occiput intense tomenté, à pilosité épaisse blanchâtre-cendré. Face noire luisante, prolongée obliquement (fig. 1 a et b), faiblement couverte d'une pruinosité blanchâtre, plus évidente dans la zone concave sous-

-antennaire. Le calus facial nasiforme, séparé de l'épistome par une creux bien marquée. Le premier article antennaire brun-foncé, le 2e et 3e brun-café; le 3e article subcirculaire, faiblement tomenté blanc. Chète antennaire pubescente.

Thorax. Noir olivâtre luisant, à pilosité blanchâtre et à deux bandes longitudinales étroites, faiblement visibles (d'en haut), formées de toment

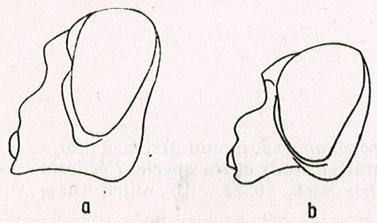

Fig. 1 — a. *Cheilosia herculana* sp. n. ♂, tête (original); b. *Cheilosia armeniaca* Stackelberg ♂, tête (après A. A. Stackelberg).

fin brun-pâle et d'une pilosité plus épaisse. Ces bandes commencent de la marge antérieure et sont visibles à peu près sur deux tiers de la longueure du mésonotum. Au devant des calus humérals deux taches de toment cendré. Scutellum à la partie apicale faiblement bordé, à pilosité pâle, plus longue sur le bord, et sans macrochètes marginales. Ailes: légèrement plus étroites qu'au *Cheilosia armeniaca* Stackelberg (fig. 2 a et b); les nervures brun-foncé; la partie basale de r_{2+3} et r_{4+5} et les nervures transverses de la partie centrale sont encadrées de brun; les superficies envoisinées aux nervures mentionnées en haut sont aussi colorés de brun mais plus claire. De sorte que les ailes ont « une tache médian ombré ». Ptérostigma jaune-bruni. Cuillerons blanches. Balanciers jaunâtres; la partie basale et le renflement plus ou moins brunies. Pattes: en grande partie noires; les extrémités apicales des f et des t et le tiers basal des t jaunes brunies. Tarses 1—3 des p_1 et p_3, à la partie apicale supérieure et à la partie inférieure jaunes-rougeâtres; tarses 1—3 des p_2 entièrement jaunes-rougeâtres, avec des épines noires microscopiques, situées a la partie inférieure. Fémurs à poils longs blanchâtres; t, surtout à la partie antérieure, à pilosité courte doré demi-couchés; tarses 1—3 des p_3, à la partie inférieure à pilosité courte et épaisse, rougeâtre, à peu près verticale.

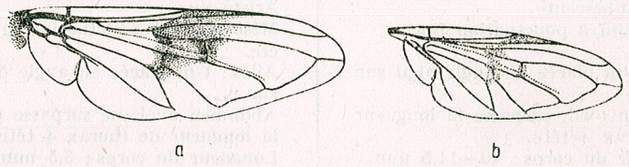

Fig. 2 — a. *Cheilosia herculana* sp. n. ♂, aile (original); b. *Cheilosia armeniaca* Stackelberg ♂, aile (après A. A. Stackelberg).

Abdomen. Ovale, olivâtre luisant, à ponctuation fine; à la partie supérieure des poils jaunes faiblement dorés, plus clairs (blanchâtres-cendrés) à la base de l'abdomen. Tergites II—IV, postéro-latéralement, à toupets couchés, ayant l'aspect des taches latérales symétriques. Sternites 3 et 4 à poils dorés, couchés sur le 3e et demi-couchés sur le 4e; sternite 2 à pilosité très longue blanchâtre, verticale. Hypopyge noir luisant, à poils blanchâtres.

FEMELLE

Semblable au mâle. Front, au niveau des antennes, quasi-égale à un tiers de la largeur de la tête, à ponctuation grossière, faiblement luisante. Triangle ocellaire et vertex noirs luisants. Pattes plus largement jaune-rougeâtre; t_1 et t_2 avec des anneaux bruns, égales à demi de la longueur du t et situé vers la partie apicale. Les nervures basales et antérieures des ailes (c, sc et r_1) brun-jaunâtre.

MATÉRIEL

Le matériel, collecté à la limite des Monts Cerna et Monts Mehedinţi (Carpates Méridionales), dans les environs de la localité Băile Herculane (160—350 m), totalisent 16 ♂♂ et 27 ♀♀ (leg. VI. Brădescu). Vallée de la rivière Cerna: 4.IX.1980, 1 ♂ 1 ♀; 15.IX. 1980, 1 ♂ 2 ♀♀. Vallée du ruisseau Feregari: 5.IX.1980, 9 ♂♂ 12 ♀♀; 18.IX.1980, 5 ♂♂ 12 ♀♀. Holotype ♂: Roumanie, Carpates Méridionales, Monts Mehedinţi, Băile Herculane, vallée du ruisseau Feregari (350 m), 5.IX.1980. Allotype ♀: les mêmes dates.

Nous dénommons la nouvelle espèce *Cheilosia herculana* après le nom de la localité Băile Herculane, zone bien-connue dans la littérature scientifique par le spécifique fito- et zoogéographique particulier de cet écosystème.

Les types et quelques paratypes sont déposés au Muséum du Delta du Danube de Tulcea; un autre nombre des paratypes au Muséum d'Histoire Naturelle « Grigore Antipa » de Bucarest.

Merodon dobrogensis sp. n.

L'espèce ne s'intègre dans nulle des clefs concernant la faune des Syrphides paléarctiques. Ayant des ressemblances prégnantes avec *Merodon flaviventris* Sack, nous présentons un tableau comparatif pour souligner les différences spécifiques:

Merodon dobrogensis sp. n.	*Merodon flaviventris* Sack
♂	♂
Corps à pilosité à trois couleurs: jaune-blanchâtre, rougeâtre et noire.	Corps à pilosité jaune-rougeâtre.
Yeux à pilosité brune.	Yeux à pilosité blanche.
Mésonotum, scutellum et pleures à pilosité jaune-blanchâtre.	Mésonotum, scutellum et pleures à pilosité rougeâtre.
Tarses 1—3 et 5 jaunes-rouilleux; le 4e brun.	Dernières articles tarsales brunâtre.
Abdomen jaune-rouilleux à taches noires verdâtres et noires pourprés-violacés, métalliques.	Abdomen jaune-rougeâtre à taches noires et brunes.
Hypopyge noir.	Hypopyge jaune-rougeâtre.
Longueur du corps: 7,75 mm.	Longueur du corps: 11 mm.
♀	♀
Semblable au ♂.	Semblable au ♂.
3e article antennaire allongé, jaune-rougeâtre.	3e article antennaire faiblement plus long que large, brun-foncé, à la partie apicale plus clair.
Longueur du corps: 8,25 mm.	Longueur du corps: 12 mm.

MÂLE

Tête. Yeux à pilosité brune. Face et front noir à reflets bleuâtres, très finement ponctuées, à pilosité jaunâtre épaisse. Triangle ocellaire à poils noirs. Antennes jaunes-rougeâtres; 3e article allongé, doucement pointu, à peu près deux fois plus long que large (fig. 3 a).

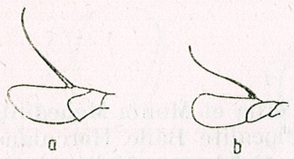

Fig. 3 — a. *Merodon dobrogensis* sp. n. ♂, antenne (original); b. *Merodon dobrogensis* sp. n. ♀, antenne (original).

Thorax. Bleuâtre luisant, finement ponctué, à pilosité dressée, jaune-blanchâtre. Ailes brunies, plus foncé vers les parties basales et antérieures; nervures de la même couleur. Cuillerons blanchâtres; balanciers/jaunes; nervures de la même couleur brunis. Pattes: f noirs, à les extrémités apicales jaunes-roujâtres; t et tarses 1—3 et 5 jaunes-rouilleux, tarses 4 bruns. Trochanters III armés d'une apophyse anguleuse, élargie à la base, égale à la longuer du 3e tarse du f_3.

Abdomen. Ovale; couleur prédominante jaune-rouilleux; ponctuation grossière, faiblement luisant; pilosité rouilleux et noire, fréquemment mélangée, mais presque exclusivement noire au tergite IV. Tergite I noir verdâtre métallique; à la partie centrale du tergite II une tache d'un noir fortement pourpré violacé métallique, bien délimitée, à l'aspect d'une coupe (fig. 4 a). Puis, la tache décrite ci-dessus présente, à travers du tergite III, un prolongement longitudinal rétreci et, après un renflement situé à la marge postérieure du tergite III, la couleur noire-pourprée violacée recouvre presque entièrement le tergite IV. Hypopyge noir luisant, à poils jaunâtres.

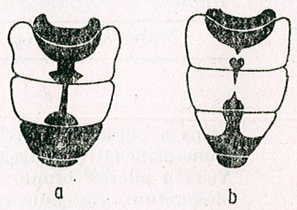

Fig. 4. — a. *Merodon dobrogensis* sp. n. ♂, abdomen (original); b. *Merodon dobrogensis* sp. n. ♀, abdomen (original).

FEMELLE

Semblable au mâle. Yeux à pilosité un peu plus courte et plus rare. Front, au niveau des antennes, presque un tiers de la largeur de la tête, modérément ponctué, luisant. Le 3e article antennaire non-pointu, un peu dilaté et arrondi à l'extrémité apicale (fig. 3b). Abdomen à pilosité rouilleux plus étendue qu'au mâle; les taches noires-pourprées violacées plus réduites (fig. 4b).

Longueur du corps: 7,75—8,25 mm.

MATÉRIEL

Holotype ♂: Roumanie, Plateau de la Dobroudja Méridionale, la forêt Hagieni — réserve naturelle complexe (altitude moyenne 100 m), département de Constanţa; 27.VIII. 1977. Allotype ♀: la même localisation; 30. VIII. 1977 (leg. Vl. Brădescu).

Nous dénommons la nouvelle espèce *Merodon dobrogensis* après le nom de la province historique Dobrogea, bien-connue dans la littérature scientifique, par le spécifique fito-et zoogéographique particulier.

Les types sont déposés au Muséum du Delta du Danube de Tulcea.

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CAULOPHRYNE BACESCUI, A NEW SPECIES OF ANGLERFISHES FROM THE PERUVIAN WATERS, EASTERN SOUTH PACIFIC (PISCES, CAULOPHRYNIDAE)

BY

ALINA MIHAI-BARDAN

The new species *Caulophryne bacescui*, previously identified under the name of *Caulophryne jordani* Goode and Bean, 1856, is described on the basis of a female specimen collected in the Peruvian waters of the Pacific Ocean during the 11th cruise of the R/V "Anton Bruun", October 1965.
Caulophryne bacescui differs from the other three species of the genus by the larger number of teeth on the two jaws, the absence of filaments along the illicium and the morphology of the esca.

During the 11th cruise of the R/V "Anton Bruun", 1965, in the northern and central waters of Peru-Chile Trench, bathypelagic and abyssal fishes were collected, many of them being deposited in the "Grigore Antipa" Natural History Museum, Bucharest (N.H.M.B.).

The specimen presented here as a new taxon was reported by Băcescu (1965) and Mayer and Nalbant (1972) as *Caulophryne jordani*, Goode and Bean, 1856.

The latter authors gave the fin rays formula, the features of the illicium along an outline drawing of the specimen and of the illicium.

CAULOPHRYNE BACESCUI sp. n.

(Figures 1–3)

Holotype: one female specimen 169.0 mm SL, "Anton Bruun" 11th cruise, Peru Trench, October 1965. The single specimen known. Cat.No 49922 N.H.M.B.

Derivatio nominis: dedicated to Prof. Mihai C. Băcescu, who took, part in the cruise and to whom this fish was given by Prof.R. Menzies scientific leader of the cruise.

Diagnosis: It differs from the other species of the genus by the larger number of teeth on the two jaws, 46 on the upper jaw (24+22), 32 on the lower jaw (17 + 15) and the absence of translucent filaments all along the illicium.

The esca is slightly dilated with a narrowing distally curved portion; distally; on its basis 2 long translucent filaments.

The rest of the filaments, 18 approximately, of different sizes, around the esca.

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Description: Body short and globulous. Head very large.

Eyes small situated about the middle of the upper jaw.

Mouth large, oblique extending beyond the eyes. Fang-like teeth, unequal, some long and slender (46 on the upper jaw, 32 on the lower jaw).

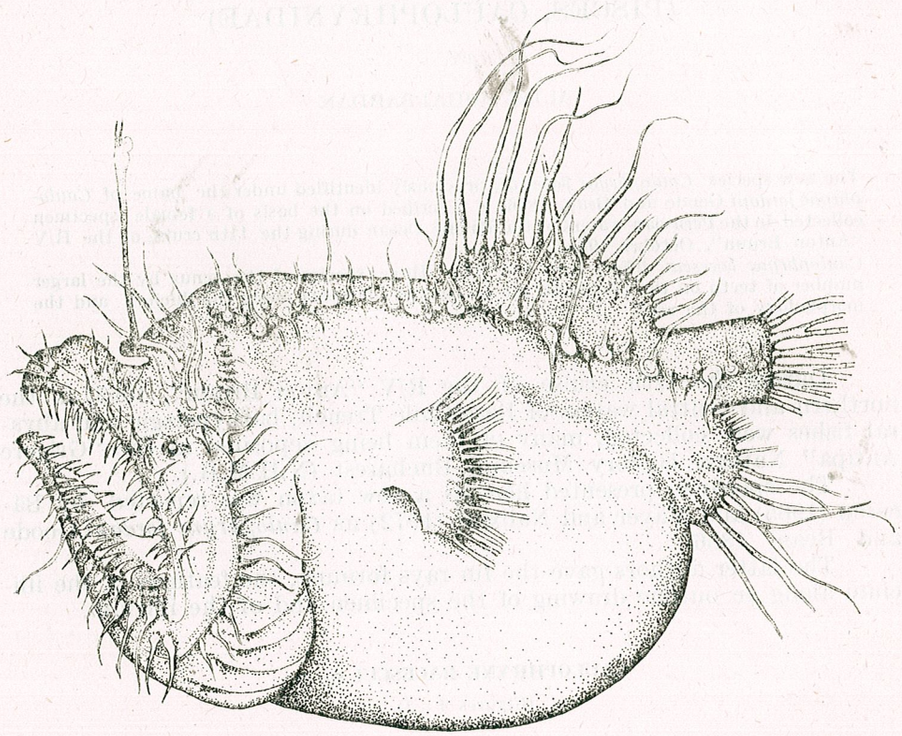

Fig. 1. — *Caulophryne bacescui* sp. n., holotype N.M.H.B. 49922, 169 mm SL

Vomerine teeth fang-like (3+3) and 16 teeth (4+4; 4+4) on the palatines (Fig. 3 A, B).

Anal fin very long situated in the posterior half of the body, near the caudal.

Insertion of pectorals at half distance between tip of snout and basis of caudal.

Illicium: anterior end of pterygiophore to which illicium is articulated very prominently towards snout forming a bulb; illicium short without illicial filaments all along; distally esca non-pigmented, slightly dilated with about 20 translucent filaments, some very small and some others very long (Fig. 3 C).

Along lateral line and lateral cephalic system there are translucent filaments, some short, some others very long. Lateral line with 33 filaments. Lateral cephalic system: frontal branch 14 filaments, maxillary branch 13 filaments, parietal branch 14 filaments, preopercular branch 12 filaments and mandibular branch 18 filaments (Fig. 2).

Fin rays formula: D (without illicium) 1 + 15, A 14, V absent, P 17—18, C II 4 II.

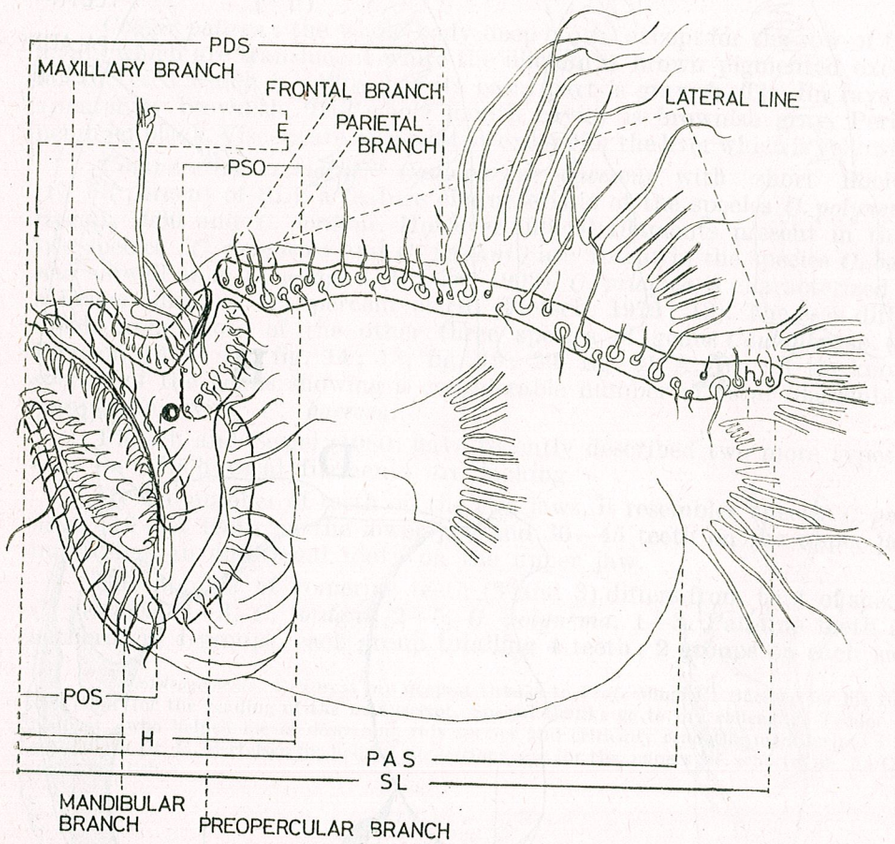

Fig. 2. — *Caulophryne bacescui* sp. n., holotype N.M.H.B. 49922, 169 mmSL. Some measurements and counts used; lateral line and lateral cephalic system. H, head; POS, preorbital space; PSO, post-orbital space; I, illicium length; E, esca length (without filaments); h, least depth of body; PDS, predorsal space; PAS, preanal space; SL, standard length.

Measurements expressed in percentages of SL (Fig. 2): head 54.43; eye 2.36; interorbital space 26.62; body depth 56.21; lower jaw 40. 23; longest tooth in lower jaw 4.73; upper jaw 36.09; illicium 17.75; esca 4.73; least depth of body 12.42; predorsal space 57.39; preanal space 82.84; length of dorsal fin base 26.62; length of anal fin base 15.97.

Inner organization: *Stomach*, very voluminous, its length 75 mm occupying 2/3 of visceral cavity. *Intestine*, syphonal, its anterior part

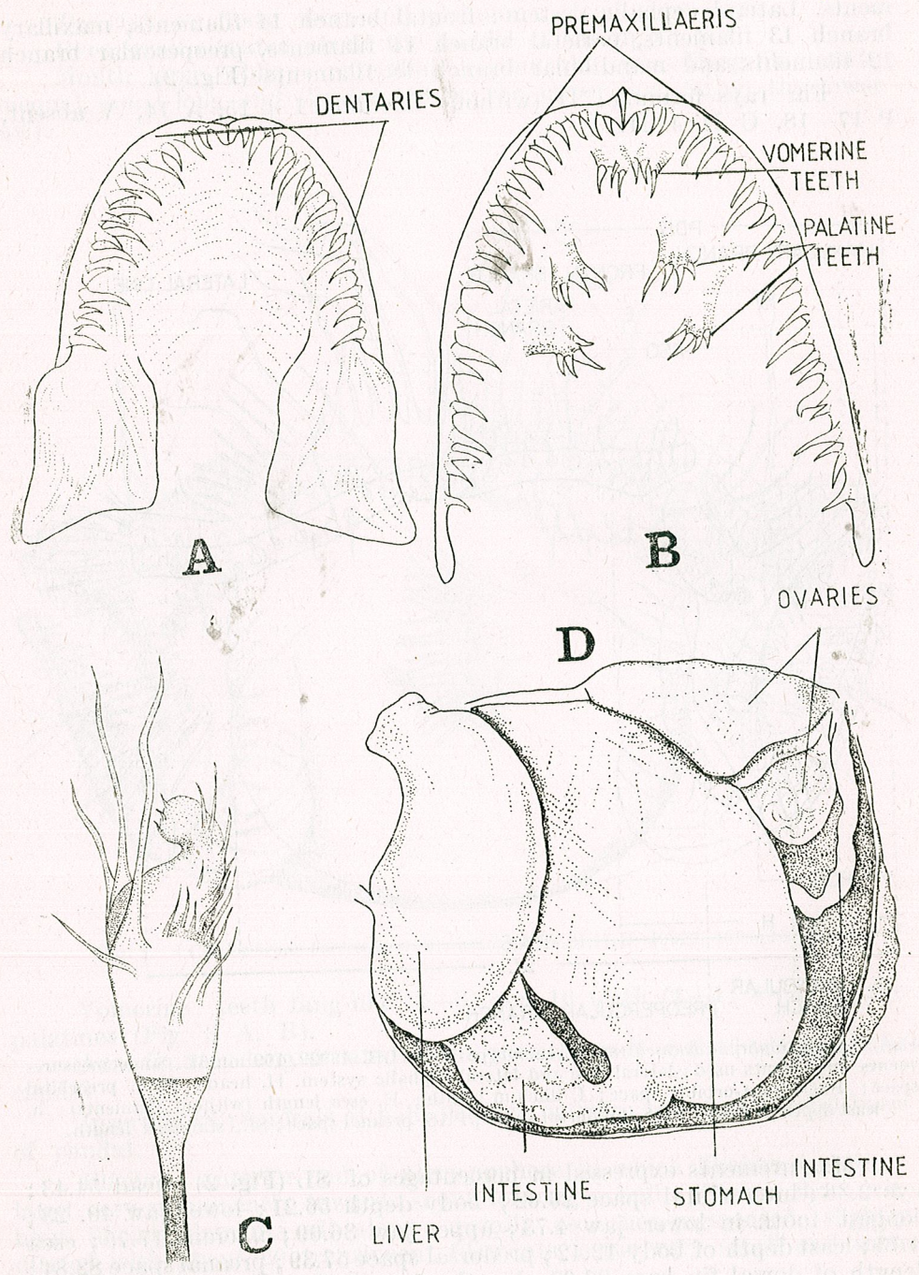

Fig. 3. — *Caulophryne bacescui* sp. n., holotype N.M.H.B 49922,169 mmSL A.-lower jaw; B.-upper jaw; C.-esca; D.-inner organization.

being more dilated; this part begins in the antero-inferior part of the stomach then ascends to the dorsal part of the cavity. The second part of the intestine forms a few loops and ends in the anal orifice. *Liver*, well developed situated in the anterior part of the stomach (62 mm length). (Fig. 3 D). *Ovaries*, form two organs included in two folded sacs; their length 44 mm. The ovarian sacs end each in the genital pore through a short duct.

Colour pattern : the whole body deep brown except for the row of filaments which are translucent white the illicium is brown pigmented except for the esca which is white, but its basal part is grayish. The fin rays are apparently brownish. The whole mouth cavity is brownish gray. Peritoneum jet black. Viscera are rose-whitish except for the liver which is yellowish.

Comparative remarks. Caulophryne bacescui with short illicium (17. 75 percent of SL), a feature characteristic of the species *C. polynema*, Regan, 1930 and *C. jordani*. However, illicial filaments present in these two species *(C. polynema* and *C. jordani)* are absent in the species *C. bacescui* as well as in *C. pelagica* (Brauer, 1902). *C. pelagica* is characterized by a longer illicium, 35 percent of SL (Pietsch, 1979 : 12). The esca differs much from those of the other three species of genus *Caulophryne* (see Pietsch, 1979 : 15, fig. 14; 18, fig. 19; 20, fig. 21 A, B), all the known species of the genus showing a considerable number of escal filaments in comparison with *C. bacescui*.

Pietsch and Seigel (1980) have recently described two more types of esca on which escal filaments are lacking.

By the number of teeth on the two jaws, it resembles species *C. polynema* (19—32 teeth on the lower jaw and 30—45 teeth on the upper jaw) but it has an additional tooth on the upper jaw.

The number of vomerine teeth (3 and 3) differs from that of species *C. pelagica*, 2—3, *C. jordani*, 2—5, *C. polynema*, 1—3. Palatine teeth are gathered in 4 groups, each group totalling 4 teeth; 2 groups on each side.

Acknowledgements. I express my deepest thanks to Prof. Mihai C. Băcescu for his guidance and for the reading of the manuscript. Special thanks go to my colleague Teodor T. Nalbant, who helped me in describing this species and critically read the manuscript. I am grateful to Dr. E. Bertelsen for helpful suggestions and for the papers he sent to me on Caulophrynid fishes.

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NEW DATA ABOUT THE MALAYAN-INDOCHINESE AFFINITIES OF THE AQUATIC FAUNA OF THE WESTERN GHATS, SOUTH INDIA

BY

PETRU M. BĂNĂRESCU and TEODOR T. NALBANT

The authors give further examples of aquatic animals from the Western Ghats having East-Himalayan, Indochinese or Malayan affinities, among the Noemacheilinae (studied by themselves), mussels and freshwater crabs (recorded in the recent literature, but not in the zoogeographical one dealing with Satpura Hypothesis). They accept a South-East Asian origin for these taxa and a westwards range extention along the Satpura mountains. The dispersal of freshwater crabs was probably the same as that of fishes and mussels.

More than three decades ago, S. L. Hora [8—10] mentioned the occurrence in south-western India, above all in the rivers of the Western Ghats, of a lot of freshwater fishes, the closest relatives of which range in the Eastern Himalayas, the Indochinese Peninsula and the Indonesian Archipelago, but are absent from the intermediate areas of the Indian Peninsula. Most examples refer to the Cyprinidae (genera *Osteochilus*, *Schismatorhynchus*, *Thynnichthys*, *Mystacoleucus*, the tribe Schizothoracini), Homalopteridae, others to Catfishes *(Silurus*, *Batasio*, *Gagata*, *Laguvia*, *Amblyceps)*; similar cases of disjunct distribution were recorded by various biologists among semi-aquatic and terrestrial animals (Turtles, Crocodiles, Snakes, Mammals, Birds, Annelid worms) and also among plants.

The available literature mentions a single example of similar disjunct distribution among the Cobitidae: the genus *Botia*, the main range of which includes southern East-Asia, Indochina, the western islands of Indonesia and the whole southern slope of the Himalayas, west of the Indus basin, one species being isolated in the Western Ghats: *B. striata* (but this example was not mentioned by Hora and the other ichthyologists who dealt with the zoogeographical problems of the Western Ghats).

Having worked, out, during the last years, the systematics of the Noemacheiline loaches, the authors could establish that *Noemacheilus*, in its present-day acceptance, is a complex of several genera, some of which are not too closely related to each others. Two of the generathe work on one of these, *Mesonoemacheilus*, is still in press) and one group of species within *Noemacheilus* in the restricted sense [1] have a disjunct South-East

[1] This genus includes, besides the *poonensis-binotatus* group mentioned here, several species in Indonesia, Malaya and the eastern half of the Indochinese Peninsula, one *(N. corica)* throughout northern India and another, *N. oxianus*, in Syr-and Amu-Dar'ya rivers, Middle Asia.

Asian — South-West Indian range, like *Osteochilus*, *Thynnichthys* and the other genera listed by Hora:

Western Ghats:	South-East Asia
Noemacheilus poonensis (upper Kistna and western coastwise rivers), *N. anguilla* (upper Kistna), *N. monilis* (upper Kaveri)	*N. binotatus* (Thailand)
Oreonectes (Indoreonectes) evezardi (upper Kistna, Godavari and western coastwise rivers), *O. (I). keralensis* (Periyar basin, western slope of Kerala State).	*Oreonectes*, other subgera (eastern slope of Kalimantan island, south-eastern China, northern East Asia).
Mesonoemacheilus triangularis, *M. pulchellus*, *M. guentheri*, *M. herrei* (in press) (all four: Kaveri River basin)-	*M. reticulofasciatus* (in press) (Brahmaputra basin in Assam).

There is a further genus of primary freshwater fishes showing the same disjunct range: *Pristolepis* of the West-Gondwanian (Inabrezian) family Nandidae, with four species in the Indochinese Peninsula and Indonesia, one of them also ranging in South India and Ceylon.

Further examples of similar disjunct ranges are furnished by the Margaritiferid freshwater mussels [7, 14], Ampullariid snails [13], freshwater crabs [2], Stoneflies [12] and Caddis Flies [6]:

Western Ghats	South-East Asia
Margaritiferid mussels	
Trapezoides prashari (Kistna basin); *Arcidopsis* (Kistna basin; related to *Trapezoides*)	*T. foliaceus* (Burma), *T. ludovicianus* (Mekong), *T. misellus* (whole Indochinese Peninsula)
Ampullariid snails	
Turbinicola saxea (Western Ghats)	*T. aperta* (hillstreams, Burma)
Freshwater Crabs	
Barythelphusa (s. str.), two species: W. Ghats, Poona, Bombay, southern tributaries of the Ganges.	*Barythelphusa (Maydelliathelphusa) lugubris*: Eastern Himalayas.
Travancoriana (South India and Western Ghats, mainly Kistna basin)	*Sartoriana* (whole Himalayas), *Liothelphusa* (Eastern Himalayas to Calcutta), *Phricothelphusa* (Burma, Tenasserim), *Lepidothelphusa*, *Thelphusula* (Kalimantan), *Adeleana* (Kalimantan, Sumatera)
Gubernatoriana (South India)	
Stoneflies (Plecoptera)	
Neoperla angulata (Ceylon), *N. moesta* (Mysore), *N. venosa* (Kodai)	*Neoperla*, many species (Indonesia, East Asia, etc).
Caddis Flies (Trichoptera)	
Moyselyella nikataruwa (Ceylon)	*M. violacea* (Assam), *M. cyanotrichia* (Burma).

The aquatic fauna of the Western Ghats, and of South India, in general, also includes a lot of endemic genera and groups of species with unknown eastern affinities; yet future studies may reveal, at least for some of them, such affinities. These are:

Cyprinidae: *Parapsilorhynchus* (Narbada River and the basins of the upper Tapti and Godavari rivers); this genus shows only a superficial

convergent similarity with the Himalayans *Psilorhynchus)*, *Horalabiosa* (former Madras Presidency), *Horadandia* (Ceylon).

Cobitidae: the genus *Nemachelichthys* (two representative species in the upper Kistna basin), *Schistura striata* (Wynaad, it may deserve generical rank).

Siluriformes: *Horaglanis* (southern part of the Western Ghats in Kerala), *Horabagrus* (same range), *Neoetropius* (northern part of the Western Ghats, probably in the Kistna basin).

Bythiniid prosobranchiata snails: *Sataria* (upper Kistna basin).

★

Hora [11] mentions the three theories proposed for explaining the occurrence of Malayan and East Himalayan biota in Peninsular India. The Himalayan glaciation theory may explain the occurrence in South India of East Himalayan (not Malayan) terrestrial animals and plants with good dispersal possibilities, but not freshwater ones that can not go from one river basin to another, except when river captures take place. Besides, the South Indian freshwater animals dealt with here are too distantly connected with their eastern relatives for assuming a quite recent (Pleistocene) dispersal. Neither is the theory of a southern route across the Indian Ocean tenable, since no direct continental contact between South India and Malaya existed in Coenozoic times, and primary freshwater animals cannot disperse through sea water. The "continuous range theory" points out the undisputable fact that disjunct distributions had sometime been continous (except for long-distance colonizations that are not possible in the case of fishes, mussels, etc.), the present-day range interruption being a consequence of geographical or climatic changes. The range-continuity between the Eastern Himalayas and the Western Ghats occurred along the Vindhya-Satpura hilly massif, as clearly demonstrated by Hora in various papers.

The "continuous range theory" is consistent with Croizat's [4] [5] biogeographical principles according to which ancestral species had wide continuous ranges; both ranges and species split later on as a consequence of geographical changes. But wide ranges are usually the result of dispersal (range extension) from a more limited area (evidently not a small "dispersal center"). In the present case, that of peculiar torrential freshwater animals from South India, it is evident that their ancestors lived initially in South-East Asia, extended their ranges along the Satpura hilly masif to the Western Ghats and later on became extinct in the former eastern part of the Satpura, that is no longer hilly now; this range interruption determined, besides distancing, the divergent evolution of the western Ghats species and their eastern relatives.

An eastern origin is quite evident for Margaritiferid mussels, a family living mainly in the Holarctis, East and South-East Asia (e.g. in areas having belonged to Laurasia), the only taxa that live in India being *Trapezoideus prashari* and *Arcidopsis*, both restricted to the Western Ghats. The fish suborder Cyprinoidei, that includes the Cyprinidae and the Cobitidae, shows a maximum diversification in East and South-East Asia; although both families also live in India and West Asia, the Cyprinidae occur in Africa, too. The Catfishes (order Siluriformes) probably

evolve, contrary to the Cyprinoidei, in the Inabrezian fragment of Gondwanaland, but soon reached South East Asia; the three families that include genera in the Western Ghats, and in South India (Siluridae, Sisoridae, Amblycipitidae) are mainly East-and South-East Asian (the second also High Asian), not occurring in India except in the Himalayas and the Western Ghats.

Freshwater Crabs are usually considered secondary freshwater animals, of rather recent marine origin; the authors who studied their dispersal history, above all Bott ([1], [3] and other papers) believe that notonly each family, but even each genus and subgenus (or species group) colonized fresh waters from distinct marine ancestors; but these families are restricted to freshwater and one may ask why did all the presumable marine ancestors of the recent genera become extinct, without having left any living offshoot in the sea. The distribution of the two taxa of freshwater crabs including representatives with South-East Asian affinities in the Western Ghats (genus *Barythelphusa*, and subfamily Liotelphusinae, the latter with six genera in the Himalayas and South-East Asia and two in the Western Ghats, see above) bears so much similarity to that of obviously primary freshwater animals such as the Cyprinidae, Cobitidae, Siluriformes and Margaritiferid mussels, that it is but natural to assume a similar dispersal, e. g. a long evolution in inland waters and dispersal by continental route.

India was initially a part of Gondwana and of its Inabrezian fragment; South-East Asia a part of Laurasia.The aquatic faunas of Laurasia and Gondwanaland were basically very different. But at least since the Cretaceous India has been a part of Asia. Its aquatic fauna includes quite few Gondwanian taxa, the most remarkable being an endemic family of Phreaticoid Isopoda (Nichollsiidae), the mussel *Pseudomulleria dalyi* that has South American affinities [14] and eventually another genus of mussels, *Hemisolsma* which, according to Starobogatov [14], belongs to the Hyriidae; the snail family, Pilidae, has a Gondwanian (Inabrezian) origin, too, but is now widely distributed also in South-East Asia. Probably, the Cichlid genus *Etroplus* is not a Gondwanian remnant, but a more recent intruder. The bulk of the aquatic fauna of India (Cypriniformes and other primary freshwater fishes, Unionid and Amblemiid mussels, most families of prosobranchiate snails, all those of Stoneflies and most or all those of Caddies Flies) belong to the Laurasian stock and gradually colonized India during the Late Cretaceous and Coenozoic times, coming from South-East Asia. The genera of fishes, mussels, etc., with East-Himalayan and Malayan affinities of the Western Ghats represent only a part of the Indian aquatic fauna of South-East Asian origin, but this part has its own dispersal history.

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QUANTITATIVE REMARKS ON IMMUNE EVOLUTION IN THE ANIMAL SERIES

BY

MIRCEA IONESCU-VARO and MIRCEA TUFESCU

Proceeding from qualitative data reported by different authors, the present work makes a quantitative analysis on the evolution of immunity in the animal series. A quantitative study of 12 immunity characters led to the detection of their different rank quality and normal-cumulative distribution. The rank, established by the different percentage ratios in the 21 taxa investigated, is plotted on polar ordinates. The tree of immune evolution obtained in this way reveals the stages traversed by the animal series in its evolution. A survey is made of the seven immune evolution levels identified.

Immunological investigations of various animal taxa carried out over the past decade have revealed the existence of characters and of specific features, of an uneven value, which sometimes are extremely difficult to correlate.

Some of the exceptionally precious synthetic works on evolution are also Hildeman and Reddy's [2] dealing with invertebrates, Machalonis and Cone's [4] with vertebrates, and E. Cooper's treatise on compared immunology [1]. In all these works, the cited syntheses included, one is struck by the lack of an all-embracing, particularly quantitative, approach to the evolution of the whole animal series and more especially to the groups of taxa having immunological affinities. What can be, in principle, objected to the purely qualitative approaches is their equating some immunological characters that are widely different in point of value and occurrence on the stage of evolution. At the same time, the lack of a quantitative method makes impossible the identification of the hiatus of immune evolutive characters in the investigated set, just because there is no criterion of appreciating their value, which makes impossible ordering them, detecting and filling in eventual gaps.

METHOD

Our analysis makes the following quantitative approach : 1) it identifies the value of immunological characters according to the rank theory, ordering the ranks and placing them within a known probabilistic distribution ; 2) it calculates the rank value of each character and identifies the hiatus of the set of characters ; 3) it builds a primary immune evolution matrix ; 4) it calculates the secondary immune evolution matrices for percent similitude (PS) and percent differences (PD) among the taxa ; 5)it builds the tree of phyletic immune evolution on polar ordinates with a

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view to pinpointing the way taxa are grouped by affinity; 6) it identifies, on this basis, the levels or stages of their immunological evolution and characterizes them.

As a matter of fact, although the matrix analysis method, the use of ranks and the building of models on polar ordinates are procedures widely employed in quantitative biology, our approach permits the direct combination of all these methods, hardly correlated so far. So, a new method is practically being developed, extremely suitable for analysing the evolution of immunity. In what follows we shall discuss this very method.

IMMUNE EVOLUTION MATRICES

1. *Primary matrix*. The works previously quoted resort to very many immunological characters which they express in terms of quality (presence-absence). But, as already stated, it is difficult to correlate the specific features identified in invertebrates and vertebrates and to distinguish some characters that have a different coverage. The set of twelve characters that could be selected are given below in their value order established by the rank identification iterative analysis (the rank is given in brackets): 1) recognition of ownness (1); 2) rejection of the xenograft (1); 3) specialized leukocytes (2); 4) rejection of the allograft (3); 5) immunological memory (4); 6) type T lymphocytes (5); 7) emergence of circulating antibodies (6); 8) emergence of organs: the thymus, the spleen (12); 9) plasmocytes (14); 10) type B lymphocytes (18); 11) lymph nodes (22); 12) Bursa Fabricii or Peyer's plates (23).

These immunological characters are given in Table 1 for 21 taxa, which represents the final form of the primary matrix built with the help of iterative analysis.

An iterative analysis of ordering the evolution of characters started from a first form of the matrix represented qualitatively by the presence-absence of the respective character. The twelve characters were ordered as they emerged so that the oldest should be on the left-hand side of table and the newest on the right one. For checking purposes we introduced the *total score* magnitude (TS) which represents the sum of all the occurrences of a character taken every time as unity.

There is one exception in the table, namely the occurrence of character 2 in *Arthropoda*, denoted with half a point since both its presence and absence in this taxon are given. TP magnitude checks the accuracy of ordering the characters in terms of their occurrence on the scale of evolution. Maximum TP values correspond to maximum antiquity, so that a decreasing TP series is obtained from the left to the right-hand side of the matrix. Exceptions make characters 4, 5, 6 which do not observe the order because their presence or absence in a number of taxa is not known. In this case, the occurrence of a character in a more primitive taxon presupposes a high occurrence probability in evolved forms for which data are still missing. So, this fact, of great biological significance, is also taken into consideration.

Table 1

Primary matrix of the ranks of the 12 characters in the 21 taxa analysed

Taxon \ Character	1	2	3	4	5	6	7	8	9	10	11	12	Total rank (amplitude)
1. Protozoa (P)	1	1	—	—	—	—	—	—	—	—	—	—	2
2. Porifera (Pf)	1	1	—	—	—	—	—	—	—	—	—	—	2
3. Coelenterata (C)	1	1	2	3	?	—	—	—	—	—	—	—	7 (5—9)
4. Annelida (Ann)	1	1	2	3	4	5	—	—	—	—	—	—	16
5. Sipunculida (S)	1	1	2	3	4	5	6	—	—	—	—	—	22
6. Mollusca (Mo)	1	1	2	3	?	—	—	—	—	—	—	—	9 (7—11)
7. Arthropoda (Ar)	1	0,5	2	—	?	—	6	—	—	—	—	—	11.5 (9.5—13.5)
8. Echinodermata (Ech)	1	1	2	3	4	5	?	—	—	—	—	—	19 (16—22)
9. Tunicata (Tu)	1	1	2	3	?	?	?	—	—	—	—	—	14.5 (7—22)
10. Cyclostomata (Cy)	1	1	2	3	?	5	6	11	—	—	—	—	31 (29—33)
11. Elasmobranchii — primitive (E_1)	1	1	2	3	4	5	6	12	—	—	—	—	34
12. — evolved (E_2)	1	1	2	3	4	5	6	12	14	—	—	—	48
13. Holostei (H)	1	1	2	3	4	5	6	12	14	—	—	—	48
14. Chondrostei (Ch)	1	1	2	3	4	5	6	12	14	?	—	—	57 (48—86)
15. Teleostei (Ts)	1	1	2	3	4	5	6	12	14	18	—	—	66
16. Dipnoi (D)	1	1	2	3	4	5	6	12	14	18	—	—	66
17. Urodela (An_1)	1	1	2	3	4	5	6	12	14	18	—	—	66
18. Anura (An_2)	1	1	2	3	4	5	6	12	14	18	(21)	—	87
19. Reptilia (R)	1	1	2	3	4	5	6	12	14	18	(21)	(22)	109
20. Aves (Av)	1	1	2	3	4	5	6	12	14	18	(21)	23	110
21. Mammalia (M)	1	1	2	3	4	5	6	12	14	18	22	23	111
Total score TS	21	20.5	19	18	14 (5)	15 (1)	14 (2)	12	9	7 (1)	4	3	

On the basis of the above analysis, an order number from 1 to 12 is obtained for the analysed characters. A second step in the iterative analysis is the determination of *the value rank of characters*. It is quite obvious that in the 1 to 12 sequence their value is increasing. One problem is to objectively identify this magnitude according to a verifiable criterion. Several attempts have been made, one of them being apparently successful. The possible distribution of characters was followed by means of the

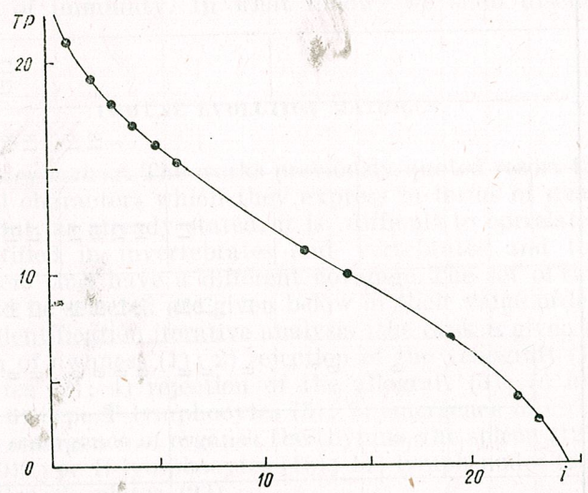

Fig. 1. — Normal ratio between the total score of each taxon (TS) on the immune evolution level and the rank of the respective characters (i); 1—12, studied characters (see text) and their rank in brackets (1—23).

different probabilistic models known. The findings have revealed that the first seven characters are ordered according to a normal-cumulative distribution in which TP is a function of rank (Fig. 1). In this case, the first and the second character behave as one, occupying the same value rank. It is quite obvious that distinguishing the ownness of the respective species requires rejection of the xenograft. Characters 2—7 are further distributed at a unity rank interval, proving to be independent. Between these characters (emergence of circulating antibodies) and the next, which is the last (occurrence of lymphoid organs: the thymus and the spleen), one notices the absence of five intermediary characters since the last is of rank 12. In this way it becomes quite clear that immunological investigations have so far overlooked the existence, in point of evolution, of a whole group of specificities. From the eighth character (rank 12) on, what is usually followed is the evolution of vertebrates. The immune evolution specificities of vertebrates (8—12) which we could make use of show a wide value scattering, with many discontinuities.

The analysis of the value rank of immune characters leads to two *preliminary conclusions:* 1) a weak knowledge of vertebrates and 2) the lack of data for transition groups hetween evolved invertebrates and ver-

tebrates, of intermediate taxa between the ancestors of Protostomia, Deuterostomia and the vertebrates. The first conclusion seems to be paradoxical given the extremely large number of immunological experiments carried out on the vertebrates. On a careful analysis, however, the speciality literature reveals a wealth of variants for a restricted group of characters. On the other hand, numerous special studies refer to related or remote taxa, but there is no analysis of compared evolution in all, or most of the groups involved. So, we may safely affirm that looking at the increased immunological complexity of vertebrates, the immune evolution data available today are scarcely sufficient compared to the information we may find on invertebrates.

2. *Secondary matrices* are two and have been calculated for percent similitude, PS, and percent differentiation, PD, which is complementary: PD = 100—PS. PS was estimated by referring twice the sum of the ranks common to the two analysed taxa to the sum of their common and different ranks. Value data for PD and PS are given in Table 2.

THE IMMUNE EVOLUTION PHYLETIC TREE

1. *Quantitative plotting on polar ordinates.* As can be seen on Table 2, the highest value falls between Protozoa — Sponges and Mammals (PD = = 96.46 %). It is notable that between Protozoa and Sponges there exists maximum immunity affinity so that both taxa record the same PD and PS values compared to the other groups. In this way, at one extremity of the representation axis there are two taxa and at the other the mammals. The axis is inscribed on the vertical, from Protozoa-Sponges to Mammals taking into account the ascending evolutionary process. The coordinates of each taxon shall be calculated in terms of the length of the axis (A), and the distribution at the two extremities (D_1, D_2), as follows:

$$d = 2A^{-1}(A^2 + D_1^2 - D_2^2) \text{ and } p = A^{-1}D_1 D_2$$

where d is the distance to the axis starting from the inferior pole and p is the distance of the point against the perpendicular line from d.

The fact that the 21 taxa analysed are inscribed on the polar ordinates conduced us to drawing up Fig. 2 on which eight major groups can be distinguished: 1) Protozoa, Sponges; 2) Coelenterata; 3) Mollusca, Arthropoda; 4), Annelida, Sipunculida, Echinodermata, Tunicata; 5)Cyclostomata, Elasmobranchii; Holostei; 6) Chondrostei, Teleostei, Dipnoi, Urodela; 7) Anura; 8) Reptiles, Birds, Mammals. The immune phyletic tree reveals both the stages of the taxa groups or singular involuted taxa. Thus, the taxa group of molluscs, arthropods, which is evidently regressing and approaching the Coelenterata, has been placed in the 2nd stage of immune evolution. On the other hand, the taxon of Holostei is noted in stage III, superior to the Chondrostei, but immunologically regressed and close to evolved Elasmobranchii. Taking into account the separation lines between stages, to the latter could be assigne dthe following amplitudes d: I = 0.15; II = 15—40; III = 40—60; IV = 60—85; V = 85—90;

Table

Secondary matrix: PS and PD indices of

PS \ PD	1.P	2.Pf	3.C	4.Ann	5 S	6. Mo	7. Ar	8. Ech	9. T_4	10 C_4
1. P	—	0	55.56	77.78	83.33	63.64	70.37	80.95	75.76	87.88
2. Pf	100	—	55.56	77.78	83.33	63.64	70.37	80.95	75.76	87.88
3. C	44.44	44.44	—	99.13	51.72	12.5	24.33	46.15	34.88	63.16
4. Ann	22.22	22.22	60.87	—	15.79	28	16.36	8.57	4.92	31.91
5. S	16.67	16.67	48.28	84.21	—	41.94	31.34	7.32	20.55	16.98
6. Mo	36.36	36.36	87.50	72.0	58.06	—	12.20	35.41	23.41	55
7. Ar	29.63	29.63	75.67	83.64	68.66	87.80	—	24.59	11.54	45.88
8. Ech	19.05	19.05	53.85	91.43	92.68	64.29	75.41	—	13.43	24
9. T_4	24.24	24.24	65.12	95.08	79.45	76.59	88.46	86.57	—	36.26
10. C_4	12.12	12.12	36.84	68.08	83.02	45	54.12	76	63.74	—
11. E_1	11.11	11.11	34.15	80	78.57	41.86	50.55	71.70	59.79	95.38
12. E_2	8	8	25.45	50	62.86	31.58	38.65	56.72	46.4	78.48
13. H	8	8	25.45	50	62.86	31.58	38.65	56.72	46.4	78.48
14. Ch	6.78	6.78	21.87	43.84	55.70	27.27	33.57	50	40.56	70.45
15. Ts	5.88	5.88	19.18	39.02	50	24	29.68	44.71	36.02	63.92
16. D	5.88	5.88	19.18	39.02	50	24	29.68	44.71	36.02	63.92
17. An_1	5.88	5.88	19.18	39.02	50	24	29.68	44.71	36.02	63.92
18 An_2	4.40	4.40	14.89	31.07	40.37	18.75	23.35	35.85	28.57	52.54
19 R	3.60	3.60	12.07	25.6	33.59	15.25	19.09	29.69	23.48	44.28
20 Av	3.57	3.57	11.97	25.4	33.33	15.13	18.93	29.46	23.29	43.97
21 M	3.54	3.54	11.86	25.2	33.08	15	18.77	29.23	23.11	43.66

VI = 90—94; VII = 94—97. Like in any macroevolutional analysis, one may notice a gradual shortening of the stages of this process, respectively a tendency of the taxa to group toward the upper extremity of evolution.

2. *Immune evolution stages* with Hildeman and Reddy [2] are assumed to be three and to comprise five levels. These stages are concentrical: 1) quasi-immune-recognition — common to all the animals; 2) cell-mediated primordeal immunity specific to evolved invertebrates and to vertebrates, featuring, like in the previous case, by allongenic incompatibility, but accompanied by specific memorization; 3) cell and humoral

2

immune evolution in the analysed taxa (1—21)

11 E_1	12 E_2	13. H	14. Ch	15 Ts	16. D	17 Am_1	18 An_2	19. R	20 Av	21 M
88.89	92.0	92.0	93.22	94.12	94.12	94.12	95.60	96.40	96.43	96.46
88.89	92.0	92.0	93.22	94.12	94.12	94.12	95.60	96.40	96.43	96.46
65.85	74.55	74.55	78.13	80.82	80.82	80.82	85.11	87.93	88.03	88.14
20	50	50	56.16	60.98	60.98	60.98	68.93	74.4	74.6	74.8
21.43	37.14	97.14	44.3	50	50	50	59.63	66.41	66.67	66.92
58.14	68.42	68.42	72.73	76	76	76	81.25	84.75	84.87	85
41.45	61.35	61.35	66.43	70.32	70.32	70.32	76.65	80.91	81.07	81.23
28.30	43.28	43.28	50	55.29	55.29	55.29	64.15	70.31	70.54	70.77
40.21	53.6	53.6	59.44	63.98	63.98	63.98	71.43	76.52	76.71	76.89
4.62	21.52	21.52	29.55	36.08	36.08	36.08	47.46	55.72	56.03	56.34
—	17.07	17.07	25.28	32	32	32	43.8	52.45	52.78	53.10
82.93	—	0	8.57	15.79	15.79	15.79	28.89	38.85	39.24	39.62
82.93	100	—	8.57	15.79	15.79	15.79	28.89	38.85	39.24	39.62
74.72	91.43	91.43	—	7.32	7.32	7.32	20.82	31.33	31.24	32.14
68	84.21	84.21	92.68	—	0	0	13.73	24.57	25	25.42
68	84.21	84.21	92.68	100	—	0	13.73	24.57	25	25.42
68	84.21	84.21	92.68	100	100	—	13.73	24.57	25	25.42
56.2	71.11	71.11	79.68	86.27	86.27	86.27	—	11.22	11.68	12.12
47.55	61.15	61.15	68.67	75.43	75.43	75.43	88.78	—	0.46	0.91
47.22	60.76	60.76	68.26	75	75	75	88.32	94.54	—	0.45
46.90	60.38	60.38	67.86	74.58	74.58	74.58	87.88	94.09	99.55	—

antibody mediated integrated immunity, emerging in vertebrates, and including also all previous specificities.

The seven stages ensuing from the quantitative analysis of the immune evolution process (Fig. 2) are presented in Table 3, as compared to Hildeman and Reddy's [2]. In what follows we give an outline of the main characters identified by us. We also abide by the basic thesis of concentric evolution in that once a character has been acquired it is being maintained, with a few exceptions, throughout subsequent stages. From this point of view, some taxa, despite their being situated in a superior

stage of evolution on a general phyletic plane, have nevertheless regressed in point of immunity, so that we placed them in an inferior stage.

I. Stage one is characterized by the capacity of organisms to respond in a positive manner to the xenograft test, being implicitly connected with the recognition of its own specificity. It comprises Protozoa and Sponges.

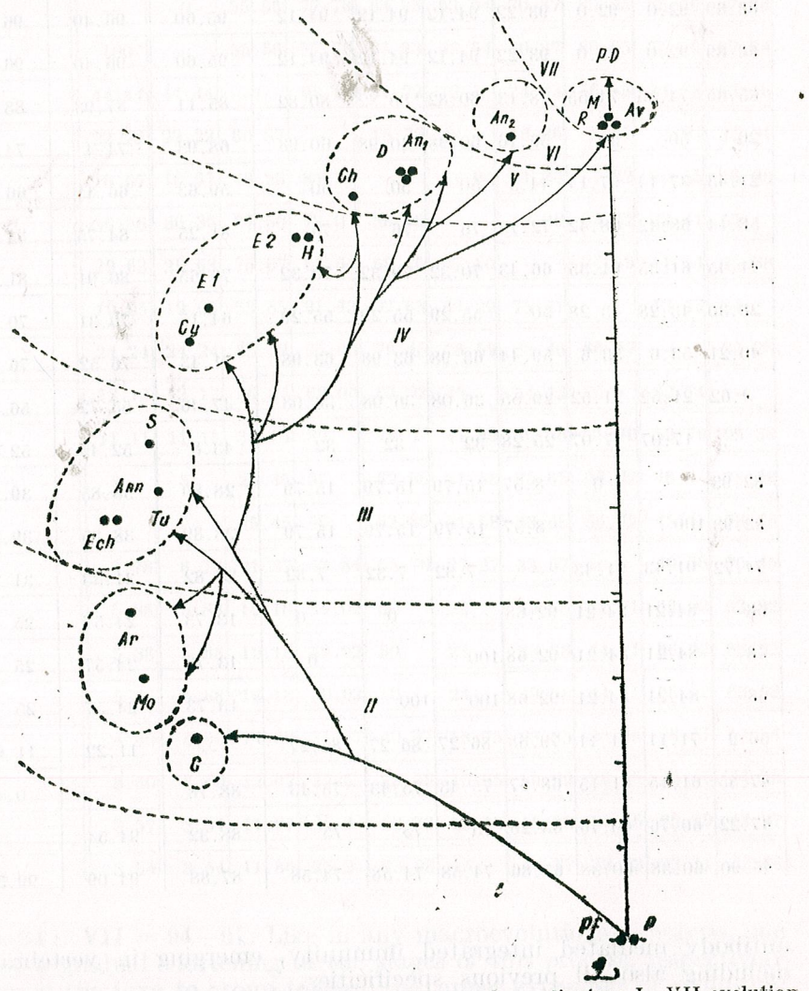

Fig. 2. — The immune phyletic tree obtained by plots on polar ordinates; I—VII evolution levels; arrows indicate phyletic relations (for abbreviations see Table 1).

II. Stage two shows the particularities of the former, adding, however, for the majority of forms, the specialization of leukocytes and the rejection of the allograft [4] and sometimes the transplanting of immunological memory [5]. It comprises the Coelenterata. At the same time,

because of an involutive process, mollusca and arthropods are placed quantitatively in this stage (Fig. 2, II bis). Mollusca show exactly the same immunity characters as the Coelenterata (Table 1), with character 5 being doubtfully identifiable and characters 6 and 7 being absent altogether, though they do occur in lower forms, e.g. Annelida and Sipunculida.

Table 3

Immune evolution

Immune evolution level		Immune specificity	Hildeman- and Reddy's conception Levels (Stages)
		character 1+2	
II	Protozoa Porifera	(*membrane receptors*)	— 1/2 (I)
		character 3+4 (+5 ?)	— 1/2
II	Coelenterata	(differentiated leukocytes)	— 2/3
III bis	Mollusca Arthropoda	(immune regression from level III) character 6 (+7)	— ? — ?
III	Annelida P Sipunculida (7) Echinodermata D Tunicata	(granulocytes + +lymphocytes (T	— 3/4 — 3/4 — 3/4 (II) — 2/3
IV	Cyclostomata Elasmobranchii	character 8 (+9) (type I specialized lymphocyte organs)	
	— primitive		— 3/4
	— evolved (g)		— ? (III)
	Holostei		— ?
V	Chondrostei Teleostei Dipnoi Urodele	character 10 (type B lymphocytes)	— ? — 4/5 — 4/5 — 4/5
VI	Anure	character 21 (lymph node pattern) character 12	— 4/5
VII	Reptilia Aves Mammalia	(type B specialized lymphoid organs)	— 4/5 — 4/5 — 4/5

Note: P = Protostomia; D = Deuterostomia

Character 7 in arthropods is similar to sipunculids, but characters 4 and 6 could not be identified and character 5 is doubtful. Noteworthy is the presence of characters 4 and 6 in Annelida which is indicative of an obvious immunological regression in arthropods.

Stage three which comprises the group of Annelida, Sipunculida, Echinodermata and Tunicata, involves the occurrence of granulocytes and type T lymphocytes [6]. We assume it to be a heterogeneous group that represents a certain level of immunity evolution wherefrom divergent lines emerged. Still more important is the fact that this group gathers two Protostomia and two Deuterostomia taxa. Yet, none of the four taxa show character 8. Out of the Protostomia, only the sipunculids display character 7, while in the two Deuterostomia taxa (Echinoids, Tunicates) it probably does exist, but has not been recognized as yet. As a matter of fact, the whole set of characters 5—7 is doubtful in Tunicata.

IV. Another heterogeneous group comprises: Cyclostomata, primitive and evolved Elasmobranchii and Holostei. They are connected immunologically by the emergence of type T specialized lymphoid organs (thymus, spleen, character 8), and the evolved taxa of the group (evolved Elasmobranchii and Holostei) show also plasmocytes (character 9). It should be noted that the Holostei seem to have regressed from immunological level V since character 10 is missing altogether.

V. This stage of immune evolution is manifest by the emergence of type B leukocytes. It comprises both the Chondrostei and Teleostei, or Dipnoi, but also the present-day primitive amphibians of the Urodela type.

VI. Formation of the lymph node patterns (character 11) in the present-day evolved golden amphibians, places them in a higher stage of immune evolution than the Urodela and the preceding taxa.

VII. The last stage features by the occurrence of type B specialized lymphoid organs. It comprises the reptiles, birds and mammals.

CONCLUSIONS

The quantitative analysis of immune evolution in the animal series has shown the following:

1) a special method of quantitative analysis which takes into account the rank value of the immunological characters on the basis of a normal distribution (Fig. 1).

2) the matrix analysis which evidences and ensures the plotting on polar ordinates of seven immune evolution levels (stages) (Fig. 2 and Table 3);

3) the rank analysis which reveals the absence of some immune evolution characters in primitive Deuterostomia, inferior to Chordata because of the focus of research on some limited specificities. At the same time, the characters discussed so far in vertebrates are scattered, whole intermediary groups being excluded.

4) in comparison with Hildeman and Reddy's [2] view on immune evolution, we assign an evolutive value to the following taxa: Mollusca, Arthropoda, Cyclostomata, evolved Elasmobranchii, Holostei, and Chondrostei. On the other hand, molluscs and arthropods are shown to have regressed in the immunological evolution from stage (level) III and Holostei from stage V into the immediately lower levels.

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ISLETS WITH INSULIN-LIKE PRODUCING CELLS IN THE INTESTINAL MUCOSA OF *MYTILUS GALLOPROVINCIALIS* (L.)*

BY

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It has been established that in the intestinal mucosa of sea mussel, *Mytilus galloprovincialis*, islet formations containing basophil and acidophil cells are present. Elective intoxication of basophil cells with alloxan (a beta cytotoxic compound) leads to a marked elevation of the haemolymphatic glucose content.

The presence of an insulin-like substance in the tissues of *Mya arenaria* was mentioned by I. B. Collip [3] in the years of insulin discovery. Despite this fact and of the works of invertebrate endocrinology agreeing that molluscs do metabolize carbohydrates [7], the physiological role of insulin [4], [11], [12], [16] and the presence of insulin-like substances and insulin-like producing cells in some molluscs have convincingly been demonstrated only in the last few years [1], [5], [6], [13], [16].

More recently, we have demonstrated [11] that the hypoglycemic effect of insulin in *Mytilus galloprovincialis* is potentiated by a glucose-inductible endogenous factor. At the same time, we have found [11] that in this species alloxan induces hyperglycemia. These observations and the above-mentioned evidence encouraged us to try to demonstrate histologically the presence of insulin-like producing cells in sea mussel, and the possible sensitivity of these cells to the beta-cytotoxic action of alloxan. Parallelly, the glucose level of the haemolymph was followed.

MATERIALS AND METHOD

Sea mussels were collected on July 28th 1976 from the low-waters of the Romanian Black Sea, littoral and were kept in aerated natural sea water at 22°C in the laboratory (Romanian Institute for Marine Research, Constanța-Agigea) for 4 days before being used in experiments. The water from the plastic tanks in which the animals were kept, was changed twice a day.

For experiments mussels of 3 to 5 cm long were used. They were divided into two groups as follows: a control group, injected with saline solution (filtered and sterilized sea water), and a group injected with saline solution containing 12.1 mg alloxan ("Austranal") for 100 g b.w. The final volume of solution (100 microliters per animal) was injected into the

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hepatopancreas *via* the ligament, by means of a microsyringe, having an adjustable needle No. 20. After 24 hours, the shells were opened following unilateral sectioning of the posterior adductor muscle. Thereafter the interpallial water was blotted by filter paper and haemolymph was collected for glucose assay. Glucose was determined by the glucoseoxidase-peroxidase method of H. A. Krebs *et al.* [10], using a VSU-2G spectrophotometer (Carl Zeiss, Jena).

Pieces from the hepatopancreatic area were dissected and fixed in Bouin's solution. After standard paraffin embedding of the pieces, the histological sections (5 μm thick) were stained by the classical Gomori technique [15]. The sections were examined and evaluated on the light microscope (magnification 10 × 40 and 10 × 90).

RESULTS AND DISCUSSIONS

Figure 1 represents the normal shape of two islets situated in the intestinal mucosa of *Mytilus galloprovincialis*. In these islets there are two different types of cells: the majority, basophil cells, have the same stain-saffinity as the insulin secreting beta cells in mammals, and a very small number of acidophil cells.

24 hours after a single injection of alloxan there appear profound phistological modifications in the aspect of the islets (Fig. 2). The basophil cells show a magnified dense nucleus and a vacuolized degranulated cytoplasm, while the acidophil cells remain unafacted by alloxan. Since the cytotoxic effect of alloxan upon the basophil cells of the islets is associated with a marked increase of glucose concentration in the haemolymph (Table 1), it is reasonable to assume that these cells have an insulin-like

Table 1

Glucose concentration in the haemolymph of *Mytilus galloprovincialis* in normal state (CONTROL) and 24 hours after administration of alloxan (ALLOXAN)

micromole glucose per 100 ml haemolymph

CONTROL	ALLOXAN	Difference %
57.89±9.06 (14)	84.89±13.06 (13)	+46.64*

The results represent means ± S. E. The number of experiments are given in parentheses. * Statistically significant difference ($P < 0.05$) as compared to the control.

producing function and therefore it is probable that they play a physiological role in the control of the carbohydrate metabolism in this species. This possibility seems to be confirmed by the findings of H. A. R. Fritsch *et al.* [5], [6] who have demonstrated by cytochemical and immunofluorescence investigations the presence of some insulin-like producing cells between the intestinal mucosa epithelial cells in *Mytilus edulis*. On the other hand, E. Plisetskaya *et al.* have recently demonstrated insulin-producing cells in the gut of the freshwater bivalve mollusc *Anodonta cygnea* and *Unio*

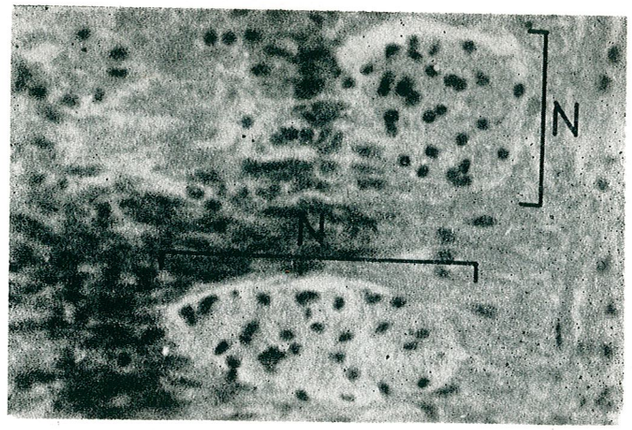

Fig. 1 — The shape of normal islets (N) in the intestinal mucosa of *Mytilus galloprovincialis* Ob. × 40.

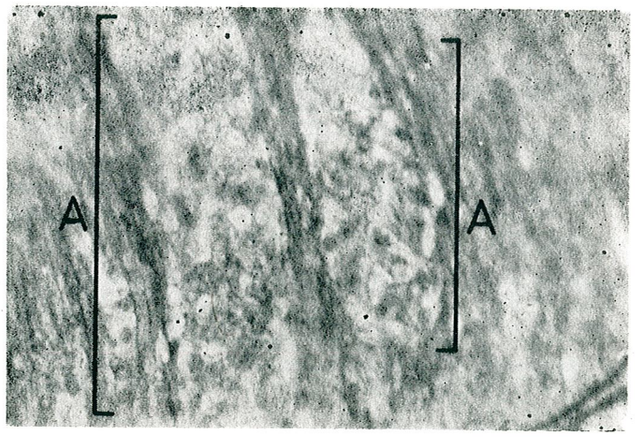

Fig. 2. — The shape of alloxan-intoxicated islets (A) in the intestinal mucosa of *Mytilus galloprovincialis* Ob. × 40.

pictorum and have found that insulin is involved in the regulation of the carbohydrate metabolism in these species [16].

It is well established that in the lower vertebrates as well as in mammals, alloxan-induced hyperglycemia and alloxan diabetes is due to the specific cytotoxic effect of alloxan upon the insulin producing beta cells of the Langerhans islets [9]. Our recent data [11] show that the concomitant administration of glucose and recrystallized glucagon-free ox insulin in *Mytilus galloprovincialis*, at 6 hours after injection, induces a greater depletion of glycemia than the administration of insulin alone, indicating a potentiation of the hypoglycemic effect of exogenous insulin by an engenous factor released by the glucose injection. In fact, it is well established that glucose is an adequate physiological secretagogue of insulin-producing islet cells [8], [2], [14], and therefore seems very possible that this sugar acts similarly upon the basophil cells of islet formations in the intestinal mucosa of *Mytilus galloprovincialis*, described here.

CONCLUSIONS

1. In the intestinal mucosa of *Mytilus galloprovincialis*, baso-and acidophil cells containing islet-like formations are present.

2. The cytotoxic effect of alloxan upon the basophil cells of islet-like formations is associated with a marked increase of glucose concentration in the haemolymph, indicating the possible role of these cells in insulin-like substance production in this species.

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L'INFLUENCE DES CONDITIONS DÉFAVORABLES DE DIAPAUSE ET D'INFESTATION PARASITAIRE SUR LES GONADES DE CERTAINS LÉPIDOPTÈRES

PAR

MARIA TEODORESCU, VIORICA TRANDABURU, VERONICA HEBEAN, ELENA PAU, DUMITRA GRUNCA

The alterations of the reproductive tissue during the diapause under low constant or variable temperatures, as well as under parasite infestation conditions have been investigated in *Antheraea pernyi* and *Philosamia ricini*. The alterations were more stable as the diapause interval was longer and the temperature more variable. These conditions abolished the gametogenesis process and facilitated the infestation of insects with parasites.

L'histologie normale et pathologique des insectes retient encore l'attention des chercheurs [7], [8]. On spécifie même quelques-unes des causes importantes qui provoquent le blocage de la gamétogenèse, telles que le déficit trophique, le déséquilibre hormonal, les conditions défavorables de la diapause, les affections parasitaires, etc. Quelques données portent sur les voies d'accès des parasites jusqu'aux cellules sexuelles [1], [4], [6], [10], [11].

Notre recherche vise à élucider, sur la base des aspects cytologiques du tissu reproducteur, s'il existe une relation entre les conditions défavorables de la diapause et le degré de sensibilisation des insectes à l'infestation parasitaire.

MATÉRIEL ET MÉTHODE

On a étudié des larves au V^e stade et des chrysalides d'*Antheraea pernyi* Guér. (Saturniidae) ainsi que des chrysalides de *Philosamia ricini* Boisd (Saturniidae) collectées pendant l'été et élevées en laboratoire, les premières jusqu'au mois de novembre et les autres jusqu'à février et mai. Ce n'est qu'aux chrysalides de *Philosamia* qu'on a analysé les modifications cytologiques pendant la période de la diapause, dans les conditions de basse température (3°C), en prélevant le matériel histologique 2 et 5 mois après l'entrée en diapause. Un autre matériel de *Philosamia* a été récolté après 4 mois de diapause à une température oscillante (—20°C—+1°C), ou après 7 mois, la température redevenant constante (3°C) au cours des trois derniers mois. On a observé dans certains cas, particulièrement sur le tégument des larves d'*Antheraea*, des zones brunes plus ou moins étendues, provoquées par l'infestation parasitaire.

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RÉSULTATS

Ci-dessous sera donnée une brève description des caractères communs de la structure normale des gonades chez les Lépidoptères étudiés.

Le *testicule*. Il est délimité à la périphérie par une capsule qui envoie vers l'intérieur des parois conjonctives, en compartimentant la gonade en tubes séminifères. Les kystes à cellules sexuelles isogènes apparaissent sous forme de massifs cellulaires dans la partie apicale des tubes ; ensuite elles acquièrent un lumen, s'élargissent, les cellules sexuelles se disposant à leur périphérie. Parmi les cellules sexuelles sont présentes les cellules nutritives qui ont le noyau plus petit que celui des gonies et émettent des prolongements cytoplasmatiques s'intercalant parmi les cellules sexuelles. Dans la phase de division, les spermatogonies ont peu de cytoplasme, qui est dense et réfringente, et un grand noyau à chromatine adhérente sur la face interne de la membrane nucléaire. Après la période de division se forment des spermatocytes du I^{er} ordre, caractérisés par une cytoplasme riche et acidophile et par un noyau plus chromatique que celui des gonies. Il est intéressant de souligner que le fuseau de la première division de maturation n'occupe pas toujours le centre de la cellule, mais se situe à la périphérie, acquérant une position perpendiculaire par rapport à celle habituelle. Les spermatocytes s'élognent souvent un peu de la thèque du kyste avec laquelle ils maintiennent encore la liaison par une excrescence cytoplasmatique allongée ; après s'être détachées elles se groupent vers le centre du kyste. A la fin de la deuxième division de matururation les spermatocytes du II^{e} ordre forment des spermatides qui sont 4—5 fois plus petites que les spermatocytes. En se transforment en spermatozoïdes, les spermatides ont au début le noyau comme une vésicule claire, ensuite il devient allongé, homogène et intensément basophile ; dans le pôle opposé au noyau a lieu l'élimination d'une quantité de cytoplasme et l'organisation graduelle de la queue du spermatozoïde. Ce développement normal de la spermatogenèse chez les larves saines subit quelques modifications en fonction de la durée de la diapause et des conditions de température.

Nous analyserons plus loin la spermatogenèse après 2 et 5 mois de diapause, à une température de 3°C et après une température oscillante.

Après deux mois de diapause, la majorité des kystes se développent normalement, quelques-uns seulement manifestant des aspects de dégradation cellulaire. Le nombre des kystes altérés s'accroît après *5 mois* de diapause. Par la rupture de nombreux kystes les cellules sexuelles qui sont

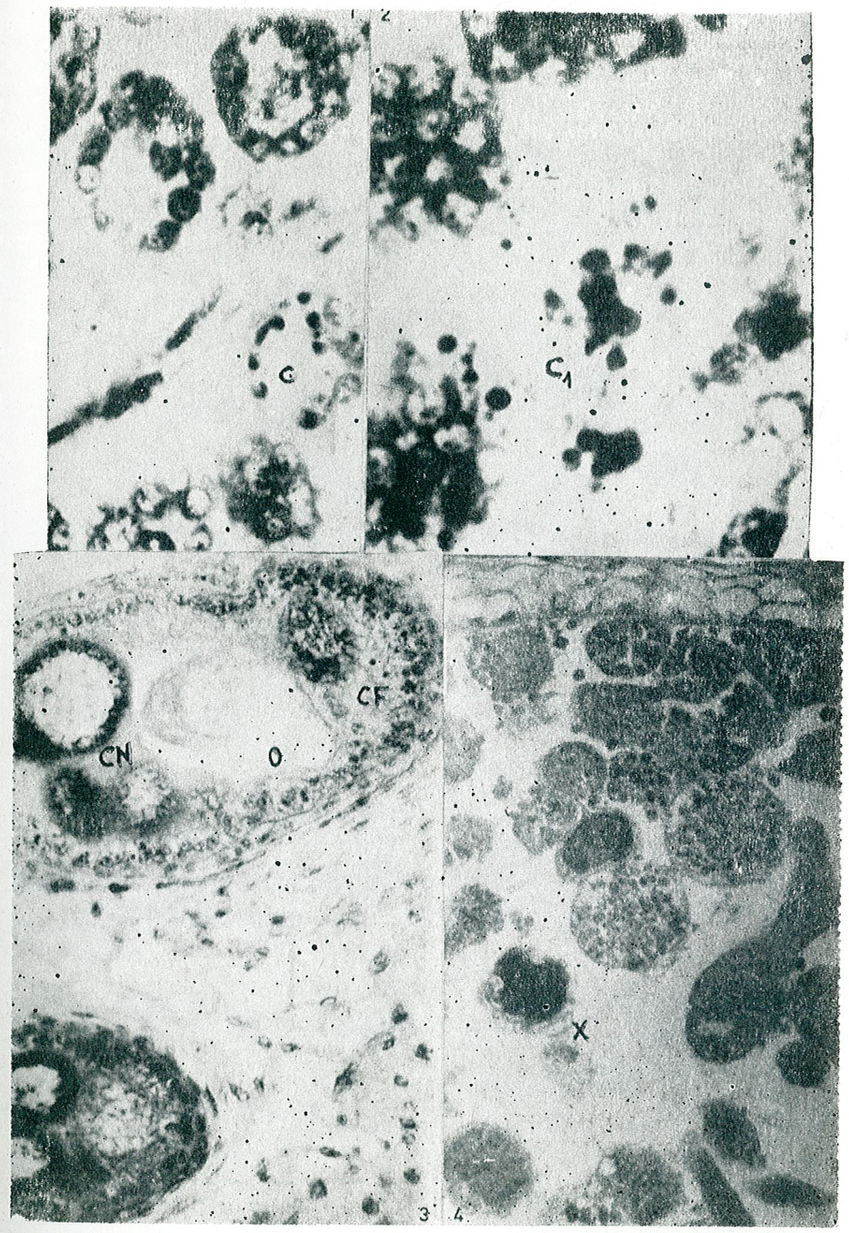
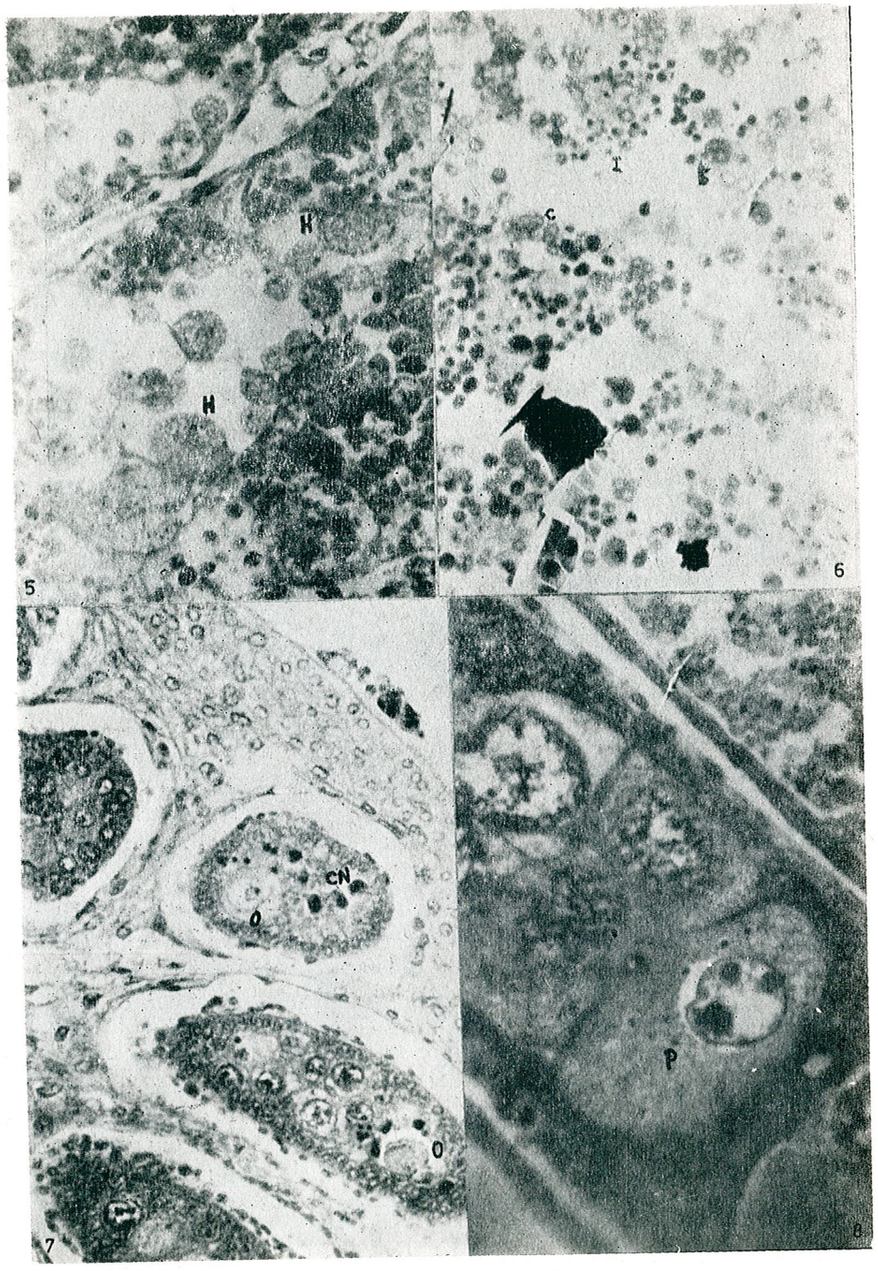


Fig. 1 et fig. 2.— Chrysalidea mascules d'Antheraea. On observe la désorganisation de quelques cellules trouvées à l'intérieur des kystes (C) ou l'involution des kystes (C) entiers provoquée par l'infestation parasitaire (C_1).

Fig. 3.— Chrysalides femelles d'Antheraea. Les parasites ont infesté la gaine des ovarioles, les cellules folliculaires (CF), et les cellules nutritives (CN), ce qui détermine l'altération des oocytes (0).

Fig. 4.— Chrysalides mascules de Philosamia sous l'influence de la température oscillante pendant 4 mois. Phases intermédiares de la destruction des kystes jusqu'à l'étape finale (X).



en cours de destruction sont mises en liberté. Bien que la température ait été gardée constante, après cinq mois la spermatogenèse est plus perturbée qu'après deux mois, dans le sens que le degré d'altération des cellules sexuelles devient stable et, donc, irréversible. L'altération des kystes se généralise si la diapause se développe pendant 4 mois dans des conditions de température basse et oscillante. Les cellules sexuelles isolées par la rupture des kystes s'amassent, confluent entre elles et engendrent par autolyse des syncitia.

Si, après 4 mois de température oscillante, le lot de chrysalides a été passé à une température constante de 3°C, pendant d'autres 3 mois, alors la topographie typique des kystes ne se conserve plus. Les tubes séminifères sont maintenant pleins de restes de kystes, de celulles sexuelles isolées ou groupées en syncitia en cours de désintégration.

En résumant les aspects de la dégradation cellulaire qui s'installe dans les conditions défavorables de la diapause, on peut affirmer qu'ils commencent par : intense vacuolisation du cytoplasme des cellules sexuelles ; dégradation de leur noyau allant jusqu'à la pycnose ; grossissement de la thèque du kyste, dont quelques cellules deviennent des phygocytes ; raréfaction des cellules dans le lumen du kyste ; désintégration des kystes ; isolement des cellules kystiques et leur autolyse individuelle ou après la formation de syncitia.

L'infestation à Nosema détermine des modifications spécifiques dans le testicule des exemplaires maintenus dans les conditions décrites ci-dessus. Les parasites, pénétrés par voie digestive ou par le système trachéolaire, arrivent à la périphérie de la gonade et peuvent être observés dans les cellules de la thèque du testicule, à la surface des kystes et même dans le cytoplasme des cellules sexuelles. On peut observer dans la même cellule un ou plusieurs parasites. La cellule entre en autolyse rapide, ou manifeste au préalable une réaction compensatoire d'hypertrophie et de vacuolisation. Par suite, le noyau est poussé à la périphérie où il se fragmente et se détruit. Le nombre des cellules du kyste diminue visiblement et, par la rupture des kystes, les cellules hypertrophiques passent dans le tube séminifère et s'autolysent.

Chez les exemplaires témoin l'*ovaire* est une glande paire et contient des ovarioles dont le tracé est contourné. Parmi les ovarioles se trouve du tissu interstitiel. L'ovariole commence par la germarium qui contient 3

Fig. 5. — Chrysalides mascules de Philosamia tenues 2 mois à 3°C. Les kystes dégradés montrent des cellules en pleine hypertrophie (H).
Fig. 6. — Chrysalides mascules de Philosamia maintenues 4 mois à température oscillante et 3 mois à temperature constante (3°C.). Après la destruction des kystes, leurs cellules libéres se disposent en cordons (C), forment des amas syncitiaux(S) ou restent isolées (I).
Fig. 7. — Chrysalides femelles de Philosamia conservées pendant 5 mois à température constante (3°C). A la suite de l'infestation parasitaire le follicule est affecte : On observe la destruction des cellules nutritives (CN) et aussi de l'oocyte(O).
Fig. 8. — Chrysalides femelles de Philosamia maintenues 4 mois à température oscillante et 3 mois à température constante (3°C). Quoique les rapports topographiques de l'oocyte de follicule soient préservés, l'oocyte manifeste des altérations à cause des parasites (P) pénétrés dans son cytoplasme.

catégories de cellules : nutritives, sexuelles et folliculaires, et continue avec le vitellarium, dans lequel les trois types de cellules entrent en rapports précis, formant les follicules ovariens. A un stade initial de développement, les cellules sexuelles du germarium ne se différencient pas des autres types de cellules. Cette difficulté est observée aussi chez d'autres insectes, telles que *Phosphuga* [12] et *Leptinotarsa* [9]. En se rapprochant du vitellarium, les oocytes s'accroissent, le noyau devient hyperchromatique et le cytoplasme acidophile. Le noyau de l'oocyte se rapproche de la zone qui prend contact avec les trophocytes par des cordons cytoplasmatiques ; il extrude dans cette direction un riche matériel nucléolaire, déterminant l'intense acidophilie de la région. Dans cette zone du cytoplasme de l'oocyte une forte concentration d'ARN a été signalée chez les Hétéroptères [16]. Dans la période de croissance, les nucléoles de l'oocyte sont plus petits, mais plus nombreux, tandis qu'à la fin de cette période il ne reste qu'un seul nucléole qui atteint une taille considérable. La membrane du noyau est généralement lisse, à l'exception de la zone voisine de la zone acidophile, dans laquelle apparaissent de petits plis de la membrane et de la région voisine au nucléole, où l'on peut observer un pli profond qui avance jusqu' au nucléole.

Le volume des trophocytes s'accroît près du vitellarium, le noyau augmente lui aussi, se charge de chromatine et apparaît intensément plissé. Les trophocytes restent isolés ou se lient par des cordons cytoplasmatiques, autant entre eux qu'avec l'oocyte. Ce dernier reçoit un cordon court et gros appartenant à plusieurs trophocytes, ou il reçoit de chaque trophocyte un cordon cytoplasmatique propre.

Les cellules folliculaires, initialement isodiamétriques et situées sur plusieurs rangs, s'allongent graduellement et se répartissent sur un seul rang autour de l'oocyte. Elle se divise fréquemment, le rythme des divisions devrant être synchrone au processus de formation des follicules. Des détails ultrastructuraux concernant le rapport entre l'oocyte et les cellules folicullaires ont été signalés par nous dans d'autres ouvrages [13].

La température constante (3°C) dérègle le processus de formation des follicules, ne déterminant leur amassement anormal qu'après une période de diapause de 5 mois. Ces dérèglements sont plus accentués après 4 mois d'influence de la température oscillante et se maintiennent aussi après 7 mois de diapause, à une température variable, au début, et constante ensuite. L'établissement des rapports normaux entre l'oocyte, les cellules folliculaires et les trophocytes devient impossible.

Les parasites infectent au début le tissu interstitiel, en produisant la destruction de nombreuses cellules ; elles passent ensuite à travers la thèque de l'ovariole et se divisent activement, particulièrement dans le germarium, occupant la place des cellules détruites à ce niveau. Les parasites attaquent les trois catégories de cellules situées dans le vitellarium, produisant la formation de vacuoles autophagiques, la vacuolisation du cytoplasme, la dégradation du noyau par pycnose, et en fin de compte, l'autolyse de l'entière cellule.

DISCUSSIONS ET CONCLUSIONS

Nos recherches confirment et complètent certaines données antérieures sur le rapport entre la t° de la croissance et le développement des insectes [2], [5], [15], particulièrement celles concernant l'influence saisonnière sur l'ovaire [12]. Nos recherches nous portent à conclure que d'altérations comparables se produisent dans le testicule aussi bien après 5 mois de diapause à 3°C, qu'après 4 mois de t° oscilante. Dans le dernier cas les altérations sont plus brutales, moins graduelles, l'autolyse s'installant de manière instantanée. L'influence de la basse t° pendant 7 mois provoque la destruction en masse des kystes avec formation de syncitia, signalées par d'autres auteurs chez d'autres insectes [3] [14]. En conclusion, la température basse et le prolongement exagéré de la diapause réduisent la fonctionnalité du testicule et dans l'ovaire déterminent aussi de troubles de la structure des rapports cellulaires et de leur transit habituel. Nos recherches coïncident avec ceux [4], [10] qui ont constaté que les parasites choisissent la voie cellulaire et non celle intercellulaire quand ils pénètrent dans le testicule et l'ovaire. L'infestation parasitaire est gonadale pour le mascule, tandis que pour la femelle elle est rencontrée aussi au long des vitelloductes.

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HISTOLOGICAL MODIFICATIONS IN THE BURSA FABRICII AND THE THYMUS INDUCED BY CYCLOPHOSPHAMIDE AND 6-MERCAPTOPURINE

BY

IOAN BABA, OVIDIU ROTARU, RODICA GIURGEA, MIRCEA GABOREANU and VIRGIL TOMA

24 hours after the third administration of the cytostatic cyclophosphamide, a follicular atrophy and a cellular depletion appear in the bursa Fabricii. After 48 hours, the bursal folds are strongly decreased and the conjunctive tissue is made evident 10 days after the last administration (on the 10th day), the tissues regenerate, so that, after 23 days, the bursa Fabricii and the thymus are completely regenerated. The cytostatic purinetol (6-mercaptopurine) has a later action. Cellular modifications appear after the 25th administration, i.e. sclerozation of the bursa and of the thymus.

The recent utilization of cytostatics in cancer therapy raised the problem of their action on different tissues, organs and mainly on the lymphopoietic syste .

The action of cyclophosphamide (CPA) was investigated in chickens and ducklings [1], [3] — [6]. The cytostatic 6-mercaptopurine (6—MP), synthetized by the Oncological Institute of Cluj-Napoca, was studied by its biochemical effect on the bursa and the thymus [2].

In this paper we present the comparative morphological changes induced on the bursa and the thymus by the administration of the both cytostatics mentioned.

MATERIALS AND METHODS

The experiment was made on the Studler-Cornish tetralinear hybrids, 5 days old, kept in laboratory conditions. The chickens were divided in the following groups: — injected i.p. with CPA (VEB Jenopharm GDR), for 1—3 days consecutively, 3 mg per 100 g b.w. daily; — injected with 6-MP for 1—28 days, 2.5 mg per 100 g b.w. daily. The control chickens received only the solvent in the same volume as the inoculated animals. The animals were killed at 1, 2, 7, 14, 21 and 28 days after the first inoculation, the thymus and the bursa collected, fixed in neutral formol 10%, included in paraffin and dyed by trichrome Masson hematoxylin-eosin, PAS and Feulgen reactions.

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RESULTS AND DISCUSSIONS

The histological examination of samples taken 24h after the first dose of CPA shows a volume diminution of the bursa follicles and a cellular depletion with a decrease of the number of blasts. In the thymus no changes were found. After 48 hours the alterations in the bursa are strongly evident. The folds are very much decreased, the follicles small and rare, the vasculo-conjunctive stroma appearing evidently between the latter. There is a clear destruction of lymphocytes. The epithelium of the bursa is high, prismatic and nonuniformly developed.

The thymic lobules are diminished and the cortical and medullar zones are slightly delimited (Fig. 1). The thymic cells are degenerated, with very rare mitoses; macrophages are seen between them.

Starting with the 10th day after the first inoculation of CPA, regenerative processes were observed in the bursa. The lymph follicles are more numerous, the vasculo-conjunctive stroma is in some places reduced. The whole structure is not uniform (Fig. 2). In the depth of the tissue, at the bases of folds, big follicles develop, full of normal lymphocytes. The thymic modifications are characterized by a weaker development of the medulla or even a vasculo-conjunctive development in this zone.

The chickens killed on the 23rd day after the first inoculation have the bursa nearly restored. Some follicles mainly in the proximity of the epithelium, are small, poor in cells and atrophied, because of the regeneration of the bystanding ones. In the thymus both zones are well differentiated; however, the medulla is smaller than in controls.

In the group injected daily with 6-MP the thymo-bursal involution is less spectacular. After the first 20 injections there is a volumetric decrease of the bursa with a diminution of the folds and a widening of its cavity. The basic structure persists but the follicles are reduced in number and volume, without any increase of the stroma. Blastic forms are very few. The epithelium is prismatic, high, not uniformly developed, and sprinkeled with microcysts. The thymus has diminished lobules, both zones being well delimited; the medulla reduced. Blastic forms and mitoses are very scarce. The vasculo-conjunctive proliferation is unimportant.

After the 25th administration of 6-MP the bursa folds are diminished, remaining as creases or villosities. A heterogeneity in form and size of the follicles is observed. There are small atrophic follicles without lymph cells. There develops an evident interfollicular (especially subepithelial) fibrosis (Figs. 3 şi 4). The bursa epithelium is high and uniformly developed, with numerous microcysts. The thymus has diminished lobules and both zones are homogenized. In the medulla there appears a conjunctive-vascular stroma. Cytologically, there are degenerated cells, cellular remainders, macrophages and an absence of blastic forms and mitoses. Comparing the action of both cytostatics on the bursa and the thymus it is obvious that CPA induces a bursal cellular depletion, very rapid and drastic, while 6-MP has a more attenuated effect. Initially, 6-MP produces an atrophy of the bursa without an exaggerated cellular depletion, but after 20—25 injections a fibrosis develops with a persistence of atrophic follicles, with lymphocytes in the latters.

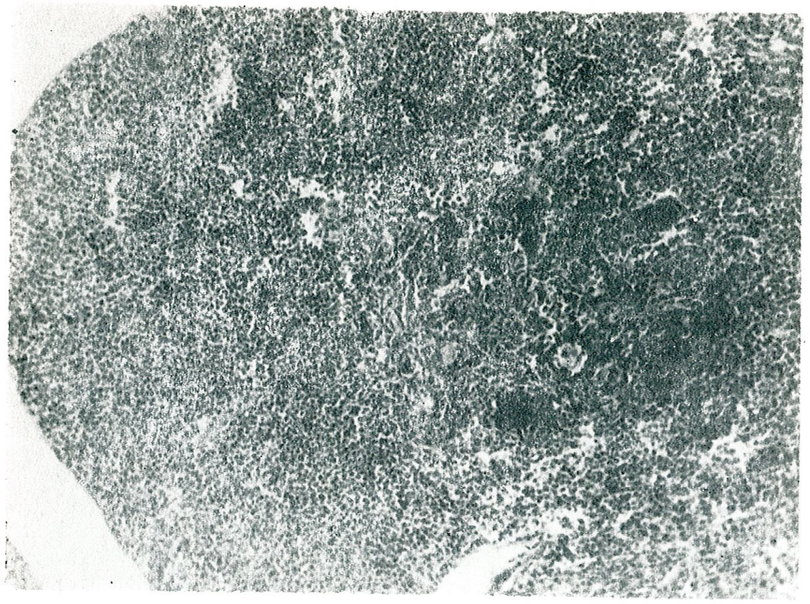

Fig. 1. — Thymus of a chicken after CPA administration shows weakly delimited cortical and medullar zones.
Trichrome Masson coloration Ob. × 100

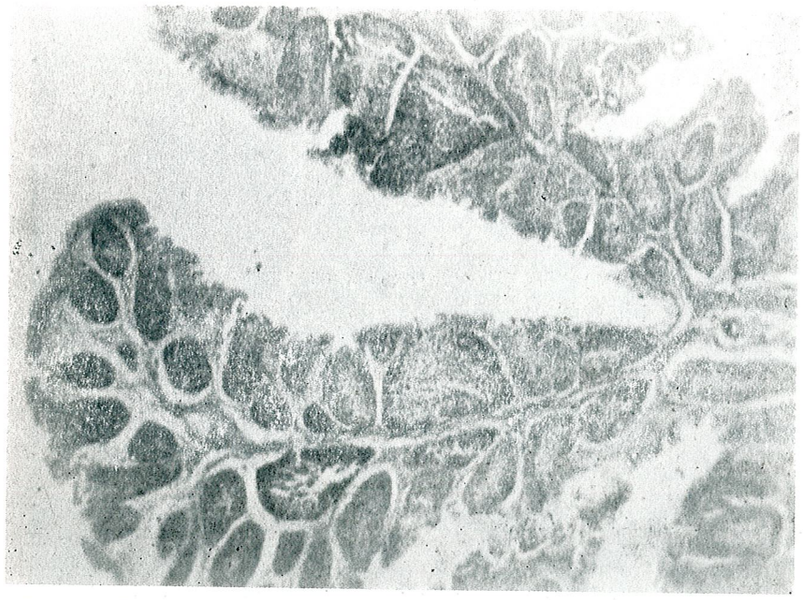

Fig. 2. — The heterogenic aspect of a bursal follicle after treatment with CPA.
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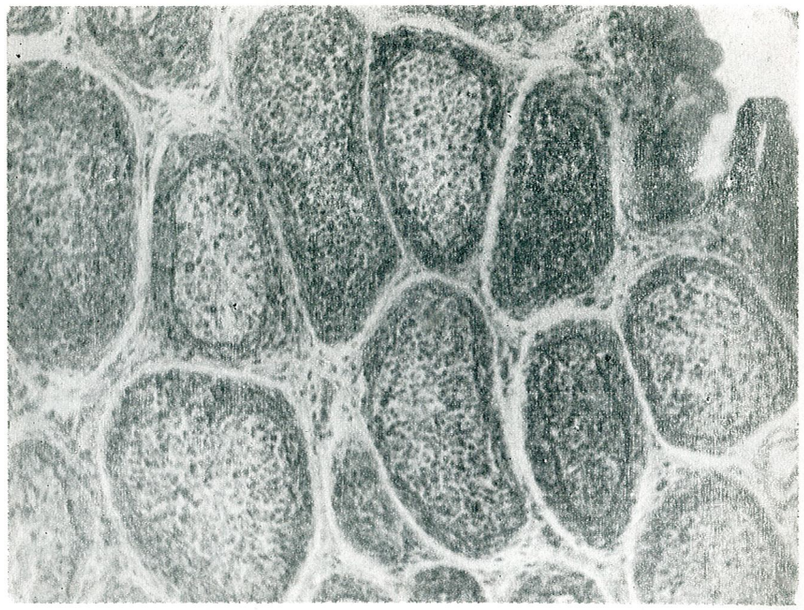

Fig. 3. — Follicular atrophy and intrafollicular fibrosis in bursal follicles after the administration of 6-MP.
Trichrome Masson coloration Ob. ×200.

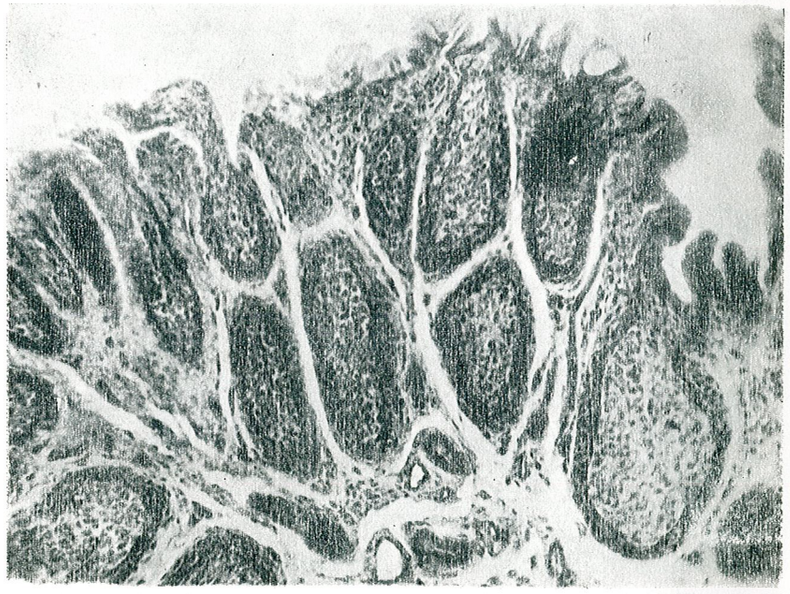

Fig. 4. — Follicular atrophy and vacuolized bursal epithelium after treatment with 6-MP.
Trichrome Masson coloration Ob. ×100

After inoculation of CPA the bursa is structurally restored beginning with the 12th day on, the process being completed by 23 days.

The reaction of the thymus for both cytostatics is slower. It involves diminishing of the medullar zone, a conjunctive-vascular reaction and homogenization of both zones.

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THE HYPOCHOLESTEROLEMIANT AND HYPOLIPEMIANT ACTION OF THE ANTIBIOTIC A.20.5

BY

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Hypocholesterolemiant and hypolipemiant effects of the polyenic antibiotic A.20.5 (given in i.m. doses of 5 mg/kg b. wt./day) have been evidenced in laboratory animals (Chinchilla rabbits) fed a mixed atherogenic regimen for six weeks. The hypocholesterolemiant effect is somewhat weaker than that of nystatin, but the hypolipemiant effect is certainly more evident. In the post-atherogenic phase the product determines a very rapid decrease of serum lipids and cholesterol concentrations.

Although the possibility of finding hypocholesterolemiant and hypolipemiant effects in other biosynthetic products is not excluded, the greatest probability of the presence of such properties remains on behalf of the polyenes, since their interactions with vegetal or animal sterols have been evidenced [4], [9], [10], [13], [14].

And yet not even in their case can we expect a similar mode of action. Some polyenes, such as filipin or nystatin have no effect upon the membranes lacking sterols or upon those in which the sterols : phospholipids ratio is 1 : 10 or even smaller [11], [12], [16].

Other polyenes, on the contrary, react with the membranes when the ratio is in favour of phospholipids [2], [3] and their lithic action could be due to the interaction with the lecithin in the membrane of the cell.

Amphotericin B has in many respects similar actions with those of nystatin, destroying the membrane structure when it is in high concentrations, and replacing their selectivity to cations with a selectivity to anions [7].

Starting from these facts we made the experiments exposed in this work by which we tried to determine whether the polyene A.20.5 has hypocholesterolemiant properties or not. The results of our studies on nystatin and CM nystatin as well as those regarding the action of the product A.20.5 upon the cell membrane were a stimulus for newinvestigations in this direction.

MATERIAL AND METHODS

The experiments were performed upon Chinchilla rabbits of the same age and with a body weight around 2 kg each. The animals were divided into two groups (of six individuals each) and fed a mixed atherogenic regimen [6]. The first group was not treated with antibiotics and to the

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other one was administered the treatment with A.20.5, too, both during the installation of the experimental atherosclerosis and in the subsequent period of recovery.

The observations were made in a period of ten weeks, as it follows: six weeks of atherogenic regimen with three stages (two weeks each) — a heavy regimen (1.55 g cholesterol/kg b. wt./day), a light regimen (0.355 g cholesterol/kg b. wt./day), and a medium regimen (0.765 g cholesterol/kg b. wt./day) — and four weeks of observations after the interruption of the atherogenic regimen. The source of cholesterol was the dry ground yolk in which the concentration was determined [5], [8]. The amount of yolk was fed in two equal portions per day, its ingestion being strictly controlled.

The treatment with A.20.5 was made during the whole observation period in i.m. injections in doses of 5 mg/kg b. wt. /day.

In order to study all these problems the animals were bled periodically and total serum lipids [8], cholesterol, and its fractions [15] were analysed.

RESULTS

A. TOTAL SERUM LIPIDS

a. In the control animals total serum lipids have an increase by 7.59 times higher than the normal value after two weeks of heavy regimen. During the light regimen a slight decrease takes place, and it is followed by a new important increase, during medium regimen. Thus, at the end of the atherogenic regimen the total concentration of lipids is by 9 .98 times higher (3143.70 mg %) than the initial one (Fig. 1, I—TL, C).

After the interruption of the atherogenic regimen a decrease in the total lipids concentration is observed, first slower, then more evident,

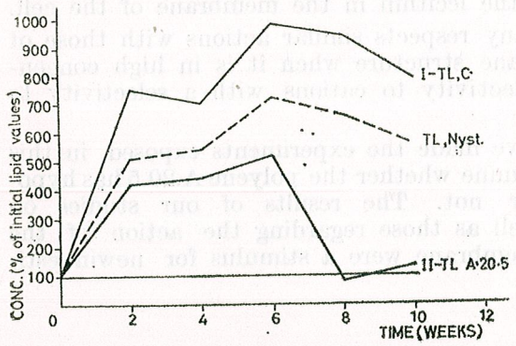


Fig. 1. — The serum variation of total lipids (TL) in animals treated with an atherogenic regimen, without antibiotic (I), with nystatin (Nyst.) or A.20.5 (II).

reaching after four weeks a value by 7.9 times higher (2488.50 mg %) than the initial one (Fig. 1, I—TL, C).

b. The treatment with A. 20,5 in the course of the atherogenic regimen leads to a reduction of total serum lipids concentration increase, much stronger than that observed in the case of nystatin treatment (Fig. 1, II—TL, A.20.5). Their concentration reaches eventually a value only by 5.26 times higher (1555.0 mg %) than the initial one.

The continuation of the treatment with A.20.5 after the interruption of the atherogenic regimen provokes a very rapid serum lipid concentration decrease. Even in the first two weeks they reach a value under the normal one (228.50 mg %). Their concentration is only 77.95 % of this one. A slight increase takes place in the following two weeks (Fig. 1, II—TL, A.20.5).

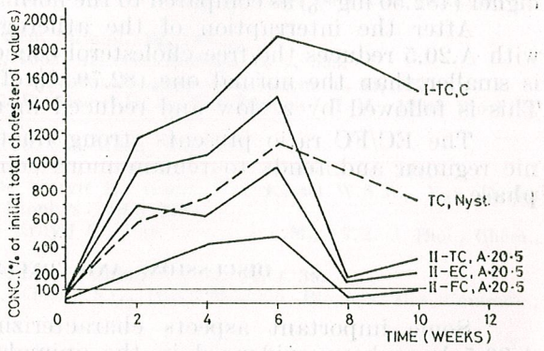
B. TOTAL SERUM CHOLESTEROL

a. In the control animals the total serum cholesterol concentration increases by 12.80 times, as compared to the initial value, after two weeks of heavy regimen. The increase goes on slowlier during the light regimen and is more evident again during the medium one. Thus, total cholesterol reaches eventually a concentration by 19.05 times higher (2771.75 mg %) than the initial one (Fig. 2, I—TC, C).

The interruption of the atherogenic regimen leads to a relatively slow decrease of total cholesterol, its concentration reaching after four weeks a value still by 15 times higher (2182.5 mg %) than the initial one (Fig. 2, I—TC, C).

b. In the animals treated with A.20.5 the total cholesterol increase is strongly influenced by the action of the antibiotic. After two weeks of heavy regimen the total cholesterol concentration is only by 9.47 times higher (964.0 mg %) than the initial one. During the light regimen the increase goes on but it is slower and more rapid during the medium regimen. Thus, finally, the value of total serum cholesterol is by 14.63 times higher (1489.0 mg %) than the initial one (Fig. 2, II—TC, A.20.5), consequently far more reduced than in control animals.

Fig. 2. — The serum variation of cholesterol in animals treated with an atherogenic regimen without antibiotic (I), with nystatin (Nyst.) or A.20.5 (II).
TC = Total cholesterol, EC = Esterified cholesterol, FC = Free cholesterol.



The continuation of the treatment with A.20.5 after the interruption of the atherogenic regimen leads to a very rapid decrease of total cholesterol which reaches a value only by 1.91 times higher (195.0 mg %) than the initial one after two weeks. Subsequently, during another two weeks a slight increase is observed (Fig. 2, II-TC, A.20.5). We point out that the action of A.20.5 upon total cholesterol during the atherogenic regimen is generally weaker than that of nystatin.

C. ESTERIFIED SERUM CHOLESTEROL

The lower efficiency of A.20.5 polyene as compared to that of nystatin in slowing down the increase of total serum cholesterol is reflected in the way this agent influences the increase of esterified cholesterol during the atherogenic regimen. After two weeks of heavy regimen esterified cholesterol concentration reaches a value by 13.92 times higher than the initial one (by 12.04 times higher in the animals treated with nystatin and by 25.60 times higher in those not treated with antibiotics).

In the two weeks of light regimen esterified cholesterol concentration decreases and becomes only by 12.16 times higher than the initial value (733.25 mg %). It increases again during the medium regimen and has a value by 19.33 times higher (1006.50 mg % than the normal one (Fig. 2, II—EC, A. 20.5).

After the interruption of the atherogenic regimen, esterified cholesterol decreases very rapidly in the first two weeks but fails to reach its normal value (295.58 % out of this). Finally, a slight increase is observed just like in the case of total cholesterol (Fig. 2, II—EC, A.20.5).

D. FREE SERUM CHOLESTEROL

The influence of the treatment with A.20.5 upon free cholesterol during the atherogenic regimen is much stronger than that of nystatin. After two weeks of heavy regimen the concentration of free cholesterol increases only by 4.81 times (239.20 mg %) as compared to the initial value (by 10.20 times in the animals treated with nystatin and by 13.10 times in those not treated). After the light regimen its concentration reaches a value by 8.77 times higher than the normal one and during the medium regimen there is only a slight increase; its value is only by 9.7 times higher (482.50 mg %) as compared to the normal one (Fig. 2, II—FC, A.20.5).

After the interruption of the atherogenic regimen, the treatment with A.20.5 reduces the free cholesterol concentration up to a value which is smaller than the normal one (82.79 % of this) in the first two weeks. This is followed by a slow and reduced increase (Fig. 2, II—FC,A.20.5).

The EC/FC ratio presents strong fluctuations during the atherogenic regimen and tends to remain more constant in the post-atherogenic phase.

DISCUSSIONS AND CONCLUSIONS

Some important aspects characterizing the specific action of A.20.5 have been evidenced in the animals fed an atherogenic regimen and treated with this antibiotic at the same time.

The increase of total serum lipids is slowed down during every stage and especially in the final one of the atherogenic regimen. Their level remains far below that in the animals treated with nystatin and very far below that one in the animals not treated. Total cholesterol increase is reduced too, as compared to that in the animals not treated, but it is not as reduced as in the case of the treatment with nystatin.

These are the reasons why the total cholesterol and other lipids ratio increases very much and continuously during the atherogenic regimen. The ratio between cholesterol fractions presents high oscillations, on the background of the increase of the esterified cholesterol relative concentration and the decrease of free cholesterol relative concentration.

The continuation of the treatment with A.20.5 after the interruption of the atherogenic regimen leads to a rapid decrease of the level of lipids and total cholesterol. There can be noticed a high level of the total cholesterol relative concentration in this case, too. The EC/FC ratio increases for a while and later it begins to decrease.

It is thus clear that A.20.5 has an action generally stronger upon lipids than upon serum cholesterol as compared to nystatin. A less specific action of free cholesterol elimination and of mobilizing esterified cholesterol seems to characterize the product. Besides all these, A.20.5 is characterized by a very strong hypocholesterolemiant and especially hypolipemiant action when administered after the interruption of the atherogenic regimen.

The results of our research recommend the further study of the possibilities of A.20.5 to be used in therapeutics as a hypocholesterolemiant agent.

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VERÄNDERUNGEN IM SPEKTRUM DER PLASMA-AMINOSÄUREN UNTER STRESSOREINWIRKUNG AN RATTEN

VON

M. L. MICHAILOV

The analysis of the free amino acid pattern in the blood plasma, when albino rats were placed under stress, produced a reduction of most of the amino acid concentrations, especially of the aspartic acid, glutamic acid and arginine and, secondly, of valine, phenylalanine, alanine and glycine. An increased reception of these amino acids in various organs may influence their level in the blood circulation.

Die freien Aminosäuren in der Blutcirculation besitzen unter physiologischen Bedingungen charakteristische, jedoch individuell unterschiedliche Konzentrationen [1], die sich unter pathologischen Einflüssen verändern können. Die Beteiligung des Aminosäureumsatzes an Stressreaktionen des Organismus wird sich in den Veränderungen des Konzentrationsniveau der freien Aminosäuren im Blutplasma widerspiegeln. Die Balance zwischen ihrem Input (Abbau von endogenen Proteinen, postabsorbtiven Zufuhr aus dem Intestinum) und Output (Regeneration der Grundstrukturen der Zellen, Syntheseprozesse der biologisch aktiven Substanzen, Deckung der energetischen Bedürfnisse durch die Glukoneogenese u.a.) wird dadurch gestört [2], [12].

Die Analyse der Veränderungen im Spektrum der Plasma-Aminosäuren unter Stressoreffekten im Tierexperiment bildete das Ziel vorliegenden Untersuchungen.

MATERIAL UND METHODIK

Bei unseren Experimenten verwendeten wir eine Population männlicher Albinoratten des Wistar Stammes im Alter von 14 Monaten mit einem Durchschnittsgewicht von 220 g. Die Tiere standen unter Standardnahrung („Altromin"). Die Versuchstiere waren über einen Zeitraum von acht Wochen als Stresstest eines Stressmusters ausgesetzt [6], [8]. In diesem kamen nach einem stochastischen System die intermittierende Bewegungseinschränkung und ein künstlich gestalteter unregelmäßig ablaufenden Hell-Dunkel-Wechsel zur Anwendung, wodurch eine Stimulierung der motorischen Spontanaktivität der Tiere erreicht wurde. Es folgte eine zweiwöchige Periode ohne Stressoreinwirkung, die der Aussiebung metabolischer Dauerschäden bei den Tieren diente. Um die Stressorwirksamkeit zu überprüfen, wurde durch eine nach Friebel und Vreden modifizierten Blutdruckmeßmethode [5] ein erhöhtes mittleres Niveau des systo-

ilschen arteriellen Blutdrucks auf 145 Torr gegenüber einem mittleren Ausgangswert von 120 Tor gefunden werden. Außerdem wurden die Tiere in Hinblick auf ihre Lernleistungen getestet. Nach diesem Aussiebungsverfahren verfügten wir über eine Versuchsgruppe von dreißig Ratten; dieser wurde aus derselben Population eine zahlenmäßig gleichstarke Kontrollgruppe gegenübergestellt.

Alle Versuchs- und Kontrolltiere wurden nach einer nächtlichen Nahrungskarenz, Wasser ad libitum, durch plötzliche Unterbrechung der Medulla spinalis getötet. Danach wurde durch Herzpunktion Blut entnommen. Bei der Vorbereitung des Untersuchungsmaterials nahmen wir eine Entweißung des Plasmas durch Sulfosalicilsäure vor. Die Bestimmung der freien Aminosäuren erfolgte mitteles Ionenaustauscherchromatographie nach Spakman, Stein und Moore [11]. In unserem chromatographischen System konnten folgende Aminosäuren erfaßt werden: Alanin, Arginin, Asparaginsäure, Glutaminsäure, Glycin, Histidin, Isoleucin, Leucin, Lysin, Methionin, Phenylalanin, Threonin, Tyrosin. Serin, Glutamin und Asparagin konnten nicht getrennt werden und wurden als gemeinsamer Peak ausgewertet. Tryptophan hatte bei dieser Methode keine reproduzierbaren Werte. Die Ergebnisse wurden mit Hilfe der Korrelationsprüfung und des t-Testes statistisch ausgewertet.

Tabelle 1

Aminosäurekonzentration in Blutplasma in μMol/l bei Ratten unter Stressorwirkung und der Kontrollgruppe; $\bar{x}$ = Mittelwerte, S = Standardabweichung, P = statistische Signifikanz.

Aminosäure	Kontrollgruppe		Stress-Gruppe		
(μMol/l)	$\bar{x}$	S	$\bar{x}$	S	P
Alanin	395	35.5	343*	36.1	0.05
Arginin	282	19	163*	16.1	0.01
Asparaginsäure	475	38.5	110*	10.5	0.01
Glutaminsäure	738.5	65	343*	26.2	0.01
Glycin	539	60.4	472*	46	0.05
Histidin	89.5	10.9	80.5	11	
Isoleucin	116.5	12.8	111	17.5	
Leucin	198.5	21.1	178.5	18	
Lysin	360	45	318	47	
Methionin	49	6.1	50	6.8	
Phenylalanin	127.5	13.3	91*	8.5	0.05
Threonin	295	30	276	33.4	
Tyrosin	30	6.9	20	5.5	
Valin	246.5	22.5	189*	19	0.05
Serin + Glutamin + Asparagin	720	30	950	146	

* statisch signifikante Differenzen

ERGEBNISSE

Tabelle I bringt die Ergebnisse der freien Aminosäuren-Analyse im Blutplasma beider Tiergruppen. Daraus sind folgende Verschiebungen im Aminosäurespektrum ersichtlich: Im allgemeinen ist eine Verminderung der Aminosäuren-Konzentrationen auffällig. Den deutlichsten Konzentrationsabfall zeigen Asparaginsäure, Glutaminsäure und Arginin. In zweiter Linie weisen Valin, Phenylalanin, Alanin und Glycin eine Verminderung ihres Niveau auf. Demgegenüber ergab die summarische Auswertung von Serin, Glutamin und Asparagin eine Konzentrationserhöhung jedoch mit großer Schwankungsbreite.

DISKUSSION

Die dargelegten Ergebnisse zeigten, daß ein erhöhter Proteinkatabolismus unter den verwendeten experimentellen Bedingungen erreicht werden kann. Es ist bekannt, daß die Stressoreffekte über die ZNS-Strukturen, Mediatoren, Hormonen sowie durch Metaboliten vermittelt werden. Man kann annehmem, daß Aminosäuren bei Stresszuständen eine erhöhte Beteiligung an der Enregieversorgung sowie den Regenerations- und Syntheseprozessen des Organismus aufweisen. Sie bilden das Hauptsubstrat für die Glukoneogenese der Säugetiere. Die stressinduzierte Glukagonaktivität stimuliert glukoneogenetische Vorgänge in der Leber. Es konnte experimentell nachgewiesen werden, daß eine Glukagoninfusion eine Verminderung der freien Aminosäure-Konzentration im Plasma infolge vermehrter Inkorporation von Aminosäuren in der Leber verursacht [4].

Kortikosteroide sorgen ebenfalls für die Aufrechterhaltung der Zulieferung von Aminosäuren in der Leber. Dieser „permessive" Effekt erfaßt sowohl die erhöhte Freisetzung der freien Aminosäuren aus den extrahepatalen Gewebe als auch ihre größere Aufnahme in der Leber sowie ihre Teilnahme an den RNS- und Proteinsyntheseprozessen [7].

Eine erhöhte Sympathikusaktivität beeinflußt die Stimulierung der Insulinsekretion durch Aminosäuren, wie Arginin, Lysin u.a. [7], [8], um kontraregulatorisch auf den Sympathikustonus zu wirken.

Der Abfall der Glutaminsäure- und Asparaginsäurekoncentration kann damit erklärt werden, daß die in metabolischen Prozessen mit Hypoxie einem großen Verbrauch unterliegen. Sie tragen bekanntlich zum Einsetzen unvollständig oxydierter Stoffwechselprodukte in den Zitronensäurezyklus bei.

Eine Argininverabreichung verursacht eine biphasische Erhöhung der Insulinsekretion sowie einen Glukagonanstieg [10]. Arginin spielt auch eine erhebliche Rolle bei der Desintoxication des Harnstoffes im Organismus; dieser Fakt ist bei Stress bedeutsam.

Valin und andere Aminosäuren mit verzweigten Seitenketten versorgen extrahepatale Gewebe, insbesondere das Gehirn, mit Aminogruppen [13]. Die Freisetzung solcher Aminosäuren ist bei erhöhter Muskelaktivität, der unsere Versuchstiere unterlagen, intensifiziert; infolgedessen kann im ZNS eine vergrößerte Utilisation dieser Aminosäuren bestehen [3].

Zusammenfassend läßt sich aussagen:

1. Stressoreffekte verursachen Veränderungen im Spektrum der Blutcirkulation. Die Mehrzahl der Aminosäuren reagiert mit Verminderung ihrer Konzentration im Blutplasma.

2. Die ausgeprägtesten Verschiebungen wurden bei Asparaginsäure, Glutaminsäure und Arginin festgestellt.

3. Die Konzentrationen von Valin, Phenylalanin, Alanin und Glycin sind ebenfalls deutlich vermindert.

4. Das Absinken der meisten freien Aminosäure-Koncentrationen in der Blutcirkulation unter Stressorwirkung ist im Zusammenhang mit der Stimulierung der metabolischen Prozesse, ihrer erhöhten Aufnahme in verschiedenen Organen und dem Energieverbrauch zu betrachten.

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DYNAMICS OF CATALASE ACTIVITY IN THE LIVER AND THE SPLEEN OF RATS FOLLOWING "WHOLE BODY" IRRADIATION WITH FAST NEUTRONS

BY

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The late effects of rat irradiation with small doses of fast neutrons (150 and 200 rad) were studied using the activity of catalase as a parameter of the modifications in the liver and the spleen collected from irradiated animals.
More or less significant fluctuations of the enzymatic activity were noticed at different times from irradiation.
On the basis of the results obtained we concluded that at 30 days after irradiation the doses of fast neutrons administered to experiment batches have induced reversible modifications in catalase activity.

INTRODUCTION

The activity of catalase following irradiation is one of the subjects much discussed in radiobiology.

"In vitro" and "in vivo" irradiations, generally in high doses, have described only the effect of electromagnetic radiations (X and gamma).

The study of the enzymatic activity of catalase, as an indicator in radioprotection and radiosensitization, arose the interest of the specialists in these fields.

Catalase activity following irradiation has been taken as a test for the evaluation of the efficiency of a radioprotector or a radiosensitizer.

As far as the catalase — irradiation relationship is concerned, there arises the question of enzyme inactivation as a direct effect of irradiation and as an indirect effect, by the appearance of increased quantities of peroxides following irradiation.

In speciality literature, papers which dwell upon the irradiation with small doses of fast neutrons are scarce. Our paper is intended to make such a study on the effects of irradiation with doses of 150 and 200 rad on the catalase activity in the liver and the spleen of rats.

MATERIAL AND METHOD

Male Wistar rats with an average weight of 150 g were "whole body" irradiated with fast neutrons (doses of 150 and 200 rad).

Single irradiation was achieved in the cyclotron by bombarding a beryllium plate with alpha particles, the dose rate being of $1.8 \times 10^8 n/cm^2 s$ (0.9 rad/s $\times$ 1μA).

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The animals were killed after 1, 2, 3, 4, 5 and 30 days from the irradiation.

The activity of catalase in the liver and the spleen homogenates was determined according to the method suggested by Lottsfeldt and collab. [7] on the basis of the Beers and Sizer's method [2] and was expressed by the velocity constant K (min^{-1}) [5].

The statistical processing of the results included :

— the standard deviation of the arithmetic mean ($s\bar{x}$),

— the standard deviation of the average difference (sd) for finding the significance of the difference (t) between the average of the control batch values and the average of the experiment batch values, considering :

— $t < 2$ nonsignificant difference
— $t > 2$ significant difference
— $t > 2.6$ obviously significant difference
— $t > 3.3$ very significant difference

RESULTS AND DISCUSSIONS

Acute "whole body" irradiation with small doses of fast neutrons in the mentioned working conditions generally induced nonsignificant modifications of catalase activity in the liver and the spleen (tables 1 and 2).

Table 1

Values of catalase activity in the liver after "whole body" irradiation with fast neutrons

Dose	Days after irradiation	Catalase activity	$s\bar{x}$	t
Control (unirradiated)	—	0.129	±0.007	—
150 rad	1	0.045	±0.005	7.36
	2	0.126	±0.015	0.18
	3	0.168	±0.009	3.33
	4	0.115	±0.011	1.15
	5	0.133	±0.013	0.30
	30	0.122	±0.008	0.63
200 rad	1	0.109	±0.014	1.48
	2	0.158	±0.017	2.00
	3	0.116	±0.015	0.95
	4	0.110	±0.008	1.68
	30	0.130	±0.016	0.08

Table 2

Values of catalase activity in the spleen after "whole body" irradiation with fast neutrons

Dose	Days after irradiation	Catalase activity	$s\bar{x}$	t
Control (unirradiated)	—	0.080	±0.005	—
150 rad	1	0.095	±0.005	2.10
	2	0.058	±0.006	3.18
	3	0.083	±0.003	0.36
	4	0.085	±0.006	0.61
	5	0.088	±0.007	0.18
	30	0.066	±0.006	1.96
200 rad	1	0.079	±0.009	0.16
	2	0.095	±0.013	1.43
	3	0.080	±0.002	0.10
	4	0.130	±0.006	7.07
	30	0.068	±0.007	1.56

In the case of the liver, very significant variations were recorded at the dose of 150 rad (table 1) on the first and the third day after irradiation ($t_1 = 7.36$; $t_3 = 3.33$) and in the spleen (table 2) on the first day (significant, $t = 2.10$) and on the second day (obviously significant, $t = 3.18$) for a dose of 150 rad and on the fourth day (very significant, $t = 7.07$) for a dose of 200 rad.

The increases and decreases of the enzymatic activity in the liver (Fig. 1) and in the spleen (Fig. 2) are characteristic of a system's answer to the action of a perturbation factor.

The variations show that the perturbation factor (irradiation with fast neutrons) induced modifications which allow the enzyme and the systems in which it is implied to recover in a very short time.

The response reactions of catalase activity following irradiation, found in the literature [1], [3], [6], [8], [9] are various and depend to a large extent on both the working conditions and the fact that two doses apparently close may belong to different domains as regards the response reaction.

Ştefan and Căpâlnă [9] found that at a dose of 1000 r the catalase activity decreases significantly in the first hours after whole body irradiation, reaching a maximum decrease after 1 hr, and reacquires normal values two hours after irradiation. At higher time intervals (8, 24 and 48 hours) significant variations as to the control have not been noticed.

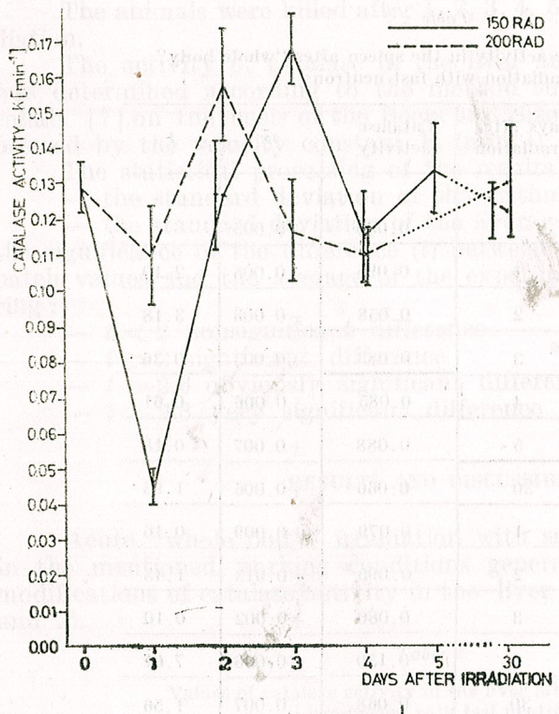

Fig. 1. — Dynamics of catalase activity in the liver after "whole body" irradiation with fast neutrons.

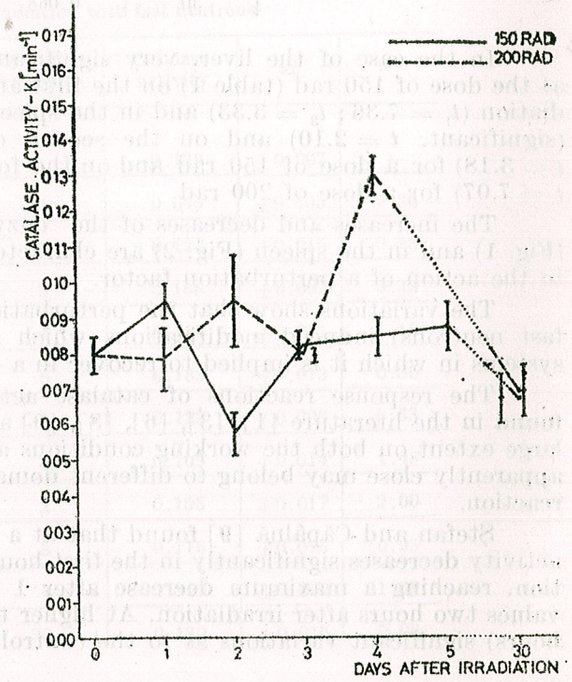

Fig. 2. — Dynamics of catalase activity in the spleen after "whole body" irradiation with fast neutrons.

The dose of 250 r [9] administered in guinea pigs induced more obvious and lengthy modifications of the catalase activity, while doses under 500 r did not induce any modification in the case of rat irradiation.

Other authors [3] remarked an activation of the enzyme after 24 hours in the case of rats irradiated with 1000 r, followed by a gradual reduction of the enzyme activity up to the third day from irradiation (90 % inhibition); starting with the fourth day, a return to normal of the catalase activity was found.

Furnică [7] established a disturbance of equilibrium between catalase activity and peroxide content following irradiation; 4 days after irradiation the return of the activity to normal parallels the normalization of the concentration in organic peroxides.

Feinstein and co-workers [4] noticed a progressive inactivation of the hepatic catalase in rats at a dose of 800 r, obtaining a maximum inactivation on the 7th day after irradiation.

Roth and co-workers [8] found in the liver of rats irradiated with 1600 r an inhibition of 30 % of the catalase activity; the inhibition maintained at this level after 12 days too.

Gorodinski and co-workers [6] showed that irradiation with 1000 rad, 2000 rad and 3000 rad induces the increase of catalase activity in muscles, 1, 2 and 7 days after irradiation, while in blood it increases after a day and decreases after 3 days.

Recently, Bartkowiak and co-workers [1] have obtained by "in vitro" gamma irradiation of bovine erythrocytes a decrease of the catalase activity at doses of 0.25—0.75 Mrad, a gradual increase at doses between 1—2 Mrad, and similar values with those of the control batch at the dose of 3 Mrad.

Our results, which show modifications at different irradiation times (1, 2, 3, 4, 5 and 30 days), can be correlated with a temporary onset of the recovery process, higher variations being possible up to a day after irradiation.

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THE CORRELATION BETWEEN THE FILTERING ZOOPLANKTON AND THE PHYTOPLANKTON

I. Recycling of the principal nutrients, nitrogen and phosphorus, by the filtering zooplankton

BY

ANGHELUȚĂ VĂDINEANU and RALUCA MUNTEAN

More than 80% of the fluctuations in the rate of nitrogen and phosphorus excretion are accounted for by the variations in individual size (W), temperature and food concentration.

The density (N) justifies less than 5% of the fluctuations in the excretion rate. Using the inductive models, it is possible to estimate, in the populations of filtrating *Cladocera* and *Copepoda*, the values of nitrogen and phosphorus excretion rates in terms of structure by sizes, temperature and food source, these value occurring in the central zone of the range delimited by the lowest and the highest values reported in literature for the same components.

The rate of the energy input in aquatic ecosystems is sometimes hard to explain due to the heterogeneity of the primary producers, the quantity of incident energy, the amount of nutrients in the environment, the temperature and the carbon dioxide concentration.

The various attempts at explaining these facts favoured the assumption that the availabilities of a high rate of primary production (energy input, except for the organic allochthonous matter) in the oligotrophic aquatic reservoirs depende on the rate at which each population within the biocoenosis returns the principal nutrients [5]. In this respect, the relationship filtering zooplankton — phytoplankton should be considered not only from the standpoint of the effect of the energetic levels of the population forming the zooplankton, but also in the light of the contribution of these populations in maintaining a certain rate of fixation of the incidental energy, through nutrient recycling and carbon dioxide production.

Although nutrient recycling by the zooplankton has been lately approached in more detail [1], [2], [6], [7],[9], [10], the results obtained so far are not sufficient to elucidate this problem. The present paper deals with the results of our studies on nitrogen and phosphorus recycling by some filtering species within the zooplankton, and on the way in which the principal environmental factors are involved in this process.

MATERIALS AND METHODS

The studies were conducted on experimental laboratory models where the filtering zooplankton consisted of three species of *Cladocera*: *Daphnia magna*, *Daphnia pulex* and *Simocephalus vetulus*, and a *Copepod*,

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Eudiaptomus gracilis; the phytoplankton consisted of the green algae *Chlorella* and *Scenedesmus*. The above species are characteristic of the plankton of aquatic reservoirs in the submergible area of the Danube and the marshes of the Danube Delta [3], [8]. In these experi ental models the algal concentration and the temperatures ranged between 0.3—10 mg of dry matter/l and 5—25°C, respectively. The fluctuation ranges of these environmental factors that affect principally the activity of the zooplankton are identical to those in the natural reservoirs. A particular emphasis was laid on the mode and extent to which nitrogen and phosphorus excretion is affected by such internal factors as population density and structure by sizes. In this respect, density (N) ranged between 5—300 individuals/l and the size (W) between 3—60/μg and 1—20/μg of dry matter in *Cladocera* and *Eudiaptomus gracilis*, respectively. For determinations of excreted nitrogen and phosphorus, groups of animals representing 10 and 20 individuals, respectively, with the same sizes, were isolated from the test jars in which the environmental factors had the values mentioned above, rinsed in distilled water and then introduced in 50 ml. Winkler jars. These jars were filled with water from the test reservoirs that was previously filtered through membrane filters with pore size of 0.45/μ. The jars thus prepared were kept for 12—14 h in the dark; the excreted ammonium was then determined by the modified method with phenol and hypochloride [12] and phosphorus by the modified method with ammonium molybdate [13].

Finally, using the multiple regression [11], the inductive models expressing the dependence rates of nitrogen and phosphorus excretion on the fluctuation of the intrinsic populational factors investigated and of the environmental factors were estimated.

RESULTS AND DISCUSSIONS

The statistical analysis of the results obtained thus far shows that there are no significant differences as to the rate of nitrogen and phosphorus excretion in the species of *Cladocera*, and this determined us to conduct the subsequent quantitative analysis for this group in an unitary way. After the analysis of the single correlations between nitrogen or phosphorus excretion and size (W), temperature (T), food concentration (H) and density (N), we decided to leave out the independent variable represented by density in the regression models, as its fluctuations in the range mentioned justify less than 5 % of the dependent variable fluctuations.

Table 1 includes the multiple regression models which allow the estimation of the specific excretion rate of nitrogen and phosphorus in the species of *Cladocera* investigated and in *Eudiaptomus gracilis* in terms of individual size, temperature and food concentration. The values of the multiple correlation coefficient (R^2) show that more than 80 % of the estimated fluctuations of the excretion rate are explained by the variations of the factors included in the models. A model resembling those described by us which allows the quantification of phosphorus excretion in *Daphnia* was developed under experimental conditions by Peters and Rigler, 1973, i.e.

$$E = 0.029\, e^{(0.039T + 0.00001C - 3.3P)} W^{-0.38}$$

where E = excretion rate of phosphorus in μg P/mg of dry matter/h, C = food concentartion expressed as number of cells/ml; W = dry weight of the animals in mg; t = temperature, P = phosphorus content of food. The authors consider that the effects of the phosphorus content in food on the excretion rate are negligible and consequently suggest the omission

Table 1

The relationship between the nitrogen and phosphorus excretion rates and temperature, food concentration and size in *Cladocera* species and *Eudiaplomus gracilis* (E_1 = μg N/mg dry weight/24 h; E_2 = μgP/mg dry weight/24 h; H = mg dry weight/l; W = mg dry weight)

	REGRESSION MODELS	R^2	F
Cladocera	$E_1 = 0.31e^{(0.082T + 0.037H)} W^{-0.46}$	0.86	12**
	$E_2 = 0.12e^{(0.078T + 0.0268H)} W^{-0.44}$	0.92	13.7**
Eudiaptomus Gracilis	$E_1 = 0.72e^{(0.084T + 0.0145H)} W^{-0.32}$	0.9	14.3**
	$E_2 = 0.2e^{(0.083T + 0.0115H)} W^{-0.253}$	0.91	18.0**

** Significant for the 99% confidence limits.

of this term in practice. It can be seen that the value of the partial regression coefficient estimated by us for the dry weight of the individuals belonging to the species of *Cladocera* (0.44) is close to the value reported by the above-mentioned authors (0.38). These values are, however, far higher than the value of the partial regression coefficient (0.253) estimated by us for the dry weight of the *Eudiaptomus gracilis* individuals. With a view to the evaluation of the extent to which each of the factors under consideration affect the nitrogen and phosphorus excretion rate, tables 2 and 3 list the results obtained in individuals of *Cladocera* sp. with the average dry weight of 8 and 40/μg and in those of *Eudiaptomus gracilis* with the dry weight of 8 and 20/μg. It can be seen that under similar temperature and food conditions, the specific nitrogen excretion rate was reduced by about 52 % at increases of the individual biomass from 8 to 40/μg dry matter in *Cladocera* but only by 25 % at biomass increases from 8 to 20/μg dry matter in *Eudiaptomus gracilis*. With regard to the dependence of the nitrogen excretion rate of food concentration (F), the data included in table 2 show that in individuals with similar sizes, grown under similar temperature conditions and on food concentration ranging from 0.3 to 10 mg. dry matter/l, the excretion rate increases by 30 % and 15 % in *Cladocera* and *Eudiaptomus*, respectively.

Moreover, it can be seen that both in *Cladocera* and *Eudiaptomus gracilis* the dependence of the rate of nitrogen excretion on temperature (T) is expressed by the coefficient Q_{10} of 2.2, this showing that the responses governed by temperature are similar.

The specific rate of phosphorus excretion (μg/mg dry matter per 24 h) changes in terms of the individual size, decreasing by 50 % in *Cladocera* at individual biomass increments from 8 up to 40/μg dry matter, and by 23% in *Eudiaptomus* at biomass increments from 8 up to 20/μg dry mater.

In animals of similar sizes grown on media containing food concentrations ranging between 0.3 and 10/mg dry matter/l it was found that the rate of phosphorus excretion increased by about 25 % in *Cladocera* and by 10 % in ***Eudiaptomus***. As revealed by the data listed in table 3, the degree of dependence of the rate of phosphorus excretion on temperature is characterized by the coefficient Q_{10} amounting to 2.2, this value being similar to that recorded for both nitrogen excretion and respiration (Vădineanu et al., Ph. thesis 1980).

Table 2

The rate of nitrogen excretion (μg N/mg dry weight/24 h) as a function of temperature, food concentration (mg dry weight/l) and size (mg dry weight)

		Cladocera		*Eudiaptomus*	*Gracilis*
T	H	$8 \cdot 10^{-3}$	$40 \cdot 10^{-3}$	$8 \cdot 10^{-3}$	$20 \cdot 10^{-3}$
20°	0.3	14.8	7.1	18	13.5
	3	16.4	7.8	19	14
	10	21.2	10	21	15.6
10°	0.3	6.5	3	7.8	5.8
	3	7.2	3.5	11.3	8.5
	10	9.4	4.5	12.6	9.4

Table 3

The rate of phosphorus excretion (μg P/mg dry weight/24 h) as a function of temperature, food concentration (mg dry weight/l) and size (mg dry weight)

		Cladocera		*Eudiaptomus*	
T	H	$8 \cdot 10^{-3}$	$40 \cdot 10^{-3}$	$8 \cdot 10^{-3}$	$20 \cdot 10^{-3}$
20°	0.3	4.8	2.4	3.6	2.8
	3.0	5.2	2.6	3.7	2.9
	10.0	6.3	3.1	4.0	3.2
10°	0.3	2.2	1.1	1.56	1.2
	3	2.4	1.2	1.62	1.3
	10	2.9	1.4	1.8	1.4

The values reported by us, as well as those estimatable according to the regression models developed for nitrogen and phosphorus excretion rates, generally range within the minimum values cited in literature — considered as basic values due to the fact that they were determined on animals starved for 48 h — and the peak values in literature — considered as artifacts because the determinations were not preceded by an adaptation to the source of food. This range of our data is accountable if we keep in mind that all determinations were preceded by a period of adaptation to the experimental conditions and the source of food.

At temperatures between 15 and 20°C, the lower values of the rate of nitrogen excretion recorded in various species of the zooplankton range between 3 and 7.5/μg N/mg dry matter/24 h [7] and the highest ones between 50 and 90/μg N/mg dry matter/24 h. [4], [7], [5]. On the basis of the data on the rate of phosphorus excretion cited by the same authors we consider that the lower values vary between 0.8—2/μg P/mg dry matter/ 24 h, and the peak ones between 13—23/μg P/mg dry matter/24 h.

The quantities of nitrogen and phosphorus returned in the circuit by the filtering species of *Cladocera* and *Copepoda* inhabiting an aquatic reservoir can be estimated by means of the regression models determined in our studies, provided that the structure and dynamics, the fluctuations of food concentration and temperature in time and space are known. Our data on nitrogen and phosphorus excretion by the filtering zooplankton demonstrate the important contribution of this component of the aquatic ecosystems in nutrient recycling and, consequently, in maintaining a certain level of primary productivity. In this respect, a pertinent answer to this problem will be obtained only after further intense studies that will also include other species of *Cladocera* and *Copepoda*, and the main species of *Rotifera*.

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MODIFICATIONS OF THE RESPONSE OF *IPS TYPOGRAPHUS* (COLEOPTERA, SCOLYTIDAE) TO THE AGGREGATION PHEROMONE IN MIXTURE WITH OTHER SUBSTANCES

N. TOMESCU[1], B.B. KIS [2], I. OPREANU [3] and LEONTINA TĂUȚAN [3]

This paper presents the results of the investigations concerning the modified response of *Ips typographus* bark beetles to the aggregation pheromone, under the influence of MCH and phenyl-ethanol. Phenyl-ethanol has determined a marked decrease of the attractivity of the principal aggregation pheromone compounds, whereas MCH had a synergic effect in 0.01 concentrations.

The efficiency of the aggregation pheromones, as a means of communication among the beetles of a population of Scolytidae is based, to a large extent, on the existence of some multifunctional compounds. Depending on their concentration, they may have an aggregative or an antiaggregative function. In the species of the *Dendroctonus* genus two such compounds have been identified : *verbenone* and MCH [1], [2], [6].

Our investigations aimed at studying the influence of MCH and phenyl-ethanol upon the activity of the major compounds of the aggregation pheromone in *Ips typographus* (ipsdienol, *cis-verbenol* and methyl--butenol).

MATERIAL AND METHOD

The investigations were performed under laboratory conditions. We used beetles coming from a natural population of *Ips typographus*. We brought trunks infested with bark beetles who were in larva stage of last age or in pupa stage. The trunks were kept in the laboratory in special boxes [5] until the complete development of the beetles. After the emergence, the adults left the spruce fir trunks and were collected in plastic bags, then kept in glass vessels at +8—10°C temperature and above 90 % relative humidity. This method of obtaining the beetles assures an adequate biological material for the laboratory experiments [4], [5]. For the bioassay we selected specimens manifesting — by going — a good physical condition. Before the bioassay, the beetles were kept in the bioassay room for 2 hours for accommodation. Seven different mixtures—formed of the major compounds of the aggregation pheromone in *Ips typographus* — were bioassayed : ipsdienol, *cis*-verbenol, methyl-butenol, alpha-pinene (a substance produced by the host plant), MCH and phenyl-ethanol. For

each variant 50 beetles were bioassayed, with two repetitions, according to the previously described method [4], [5]. The substances were delivered at a constant rate of 2 microlitres/hour by means of a mechanically worked microsyringe.

RESULTS AND DISCUSSIONS

The mixtures of the tested substances in *Ips typographus* were different according to the presence or the absence of phenyl-ethanol and MCH, this last one being in various concentrations (table 1). Ipsdienol, *cis*-verbenol and alpha pinene were put in the same proportion and methyl-butenol in a 10 times bigger amount.

The published data show that this compound may be found in bigger quantities in the composition of the natural pheromone [1].

Table 1

Variants of the concentration of aggregation pheromone tested in *Ips typographus* combined with alpha-pinene, MCH and phenyl-ethanol

	I	II	III	IV	V	VI	VII
Ipsdienol	1	1	1	1	1	1	1
Cis-verbenol	1	1	1	1	1	1	1
Methyl-butenol	10	10	10	10	10	10	10
Alpha-pinene	1	1	1	1	1	1	1
Phenyl-ethanol	—	1	1	1	1	1	—
MCH	—	—	0.001	0.1	1	1.1	0.01

Excepting variant II, the females responded in a higher percentage than the males to all the other mixtures. These results are explained by the structure of the *Ips* species populations, where the sex ratio is by far in favour of the females, a rate determined by the males' polygamous reproduction behaviour. One can infer that the aggregation pheromone in *Ips* plays a part both in the aggregation process and in the achievement of a certain proportion between the males and the females, corresponding to their reproduction behaviour. There are great differences concerning the activity of the tested mixtures. The lowest attractivity was seen in variant II where, besides the principal components of the aggregation pheromone, phenyl-ethanol was present, too. Based on the observations on the beetles' response behaviour, we are inclined to believe that phenyl-ethanol acts rather as an inhibitor than as a repellent. During the passing of the substance carrier air current, the beetles did not show a repulsion reaction, which would have determined their moving off the source. They continued to shift at random on the arena of the olfactometer.

MCH, in all the variants, had a synergic effect in comparison with the variants where it was absent. But, if we compare the activity of the variants having MCH, we see that it decreases as the MCH concentration increases. At the same time one can see that in variant V — where MCH was in the same proportion as ipsdienol and *cis*-verbenol — the males' response decreased in a much greater percentage in comparison with that of the females. This is also confirmed by the results obtained by the testing of variant VI, where the MCH proportion increased to 1.1. By testing variants III and IV we obtained close values of the response both in males and females. The four variants (III, IV, V, VI) were composed of the same substances, distinguishing themselves only by the different concentration of MCH. The different values of the males' and females' responses allow us two observations concerning the properties of the aggregation pheromones, as complex ones, composed of several substances. First of all, one can see that the attractivity of the pheromone is much influenced also by the proportion of substances, and not only by their presence or absence. Secondly, the modifications of the proportion of substances change the pheromone attractivity, differently in the two sexes. In this case, MCH in proportion of 1 :1 and 1.1 : 1 towards ipsdienol and *cis*-verbenol respectively, determines a much more important decrease of the response in the males than in the females. In both sexes the greatest attractivity was observed in variant VII, where MCH was present in a proportion of 0.01 : 1 and phenyl-ethanol was absent. These results show once more that phenyl-ethanol has an inhibitory part in the response of *Ips typographus* to the aggregation pheromone, and MCH, in reduced proportions, has a synergic part.

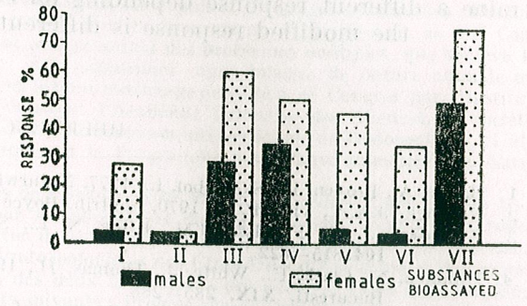

Fig. 1. — The response of the bark beetles *Ips typographus* at the major aggregation pheromone compounds in mixture with phenyl-ethanol, MCH and alpha-pinene.

CONCLUSIONS

The investigations performed in the laboratory on the attractivity of the aggregation pheromone in *I. typographus* allow the following conclusions :

— MCH and phenyl-ethanol, substances which have not been identified in the aggregation pheromone with *I. typographus*, modify the beetles' response to the own pheromone;

— phenyl-ethanol has an inhibitory action when mixed with the aggregation pheromone compounds;

— in reduced concentrations, MCH has a synergic action, increasing the aggregation pheromone attractivity. As the MCH concentration increases, its synergic effect diminishes;

— the modifications of the concentration of mixed substances determine a different response depending on the concentration;

— the modified response is different in the two sexes.

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[3] *Institute of Chemistry, Cluj-Napoca, Dr. Donath, 65—103*
Romania

LE VI[e] CONGRÈS INTERNATIONAL DE PROTOZOOLOGIE
Varsovie, Pologne, 5—11 juillet 1981

Ayant coïncidé avec l'anniversaire de vingt ans depuis le I[er] Congrès, organisé à Prague, Tchécoslovaquie, par l'initiative du protozoologiste bien connu, Otto Jirovec, ce VI[e] Congrès a pleinement démontré le puissant intérêt actuel des problèmes multiples que soulève la connaissance biologique approfondie des organismes unicellulaires de nature animale ou végétale. C'est ce qui explique également le haut patronage accordé à ce Congrès par l'Institut Nencki de Biologie expérimentale à Varsovie, l'Académie Polonaise des Sciences, la Société zoologique de Pologne, et de l'étranger par la Commission internationale de Protozoologie, l'Union internationale des Sciences biologiques et le Programme pour l'environnement des Nations Unies.

Déroulés dans les amphithéâtres de l'Académie de Musique « Frédéric Chopin », les travaux du Congrès furent inaugurés par les allocutions de son président, le prof. Stanislaw Dryl, du président Aleksander Gieysztor de l'Académie Polonaise des Sciences, du président R. Barclay McGhee de la Société des Protozoologistes des États-Unis d'Amérique et des secrétaires Adam Urbanek et John J. Lee des deux institutions précédentes. Dans deux sessions plénières, ont été présentés les rapports suivants : Progrès récents sur la culture *in vitro* des protozoaires parasites (W. Trager, New York); Variation intraspécifique et concept de l'espèce chez les protozoaires (J. I. Poljansky, Leningrad); La Protozoologie en Pologne, passé et présent (L. Kuznicki, Varsovie); Ingénierie génétique chez *Tetrahymena* (P. J. Bruns, Université Cornell, U.S.A.); Structures microtubulaires (J. B. Tucker, Université St. Andrews, Ecosse); Adaptation des membranes chez *Tetrahymena* (Y. Nozawa, Université Gifu, Japon). Une troisième session plénière a été consacrée à la discussion entre de nombreux spécialistes des relations phylogénétiques parmi les protozoaires (président B. M. Honigberg, Université Massachusetts, U.S.A.).

Des six symposiums, trois ont développé des sujets liés à des rapports précédemment cités, tandis que les trois autres ont porté sur les domaines suivants : Organites cytoplasmiques, mitochondries, kinétoplastes, hydrogénosomes, glycosomes (président M. Müller, New York); Bases ultrastructurales et moléculaires de la motilité (président K. Wohlfart-Bottermann, Bonn); Relations symbiotiques (président A. Soldo, Miami, U.S.A.).

Enfin, plusieurs centaines de communications, dont les résumés ont formé un volume de 418 pages, furent partagées entre les huit sections suivantes : I) Variation, cycles évolutifs, systématique et phylogénie des protozoaires; II) Génétique et morphogenèse, dédiée à la mémoire de T. M. Sonneborn; III) Analyse antigénique et immunogenèse; IV) Action des agents externes et chimiques; V) Motilité et comportement, dédiée à Th. L Jahn; VI) Ecologie des protozoaires libres; VII) Ecologie des protozoaires parasites en relation avec leurs hôtes; VIII) Organites cytoplasmiques et métabolisme.

Une hospitalité parfaite, cordialement régie par le président S. Dryl, le vice-président L. Kuznicki, le secrétaire général S. L. Kazubski et leurs aimables collaborateurs, a assuré une heureuse effusion scientifique entre les participants et un indéniable succès d'ensemble de ce congrès. Vu l'importance de la protozoologie pour l'écologie humaine dans les pays tropicaux menacés de graves maladies dues aux protozoaires pathogènes, le prochain congrès a été projeté d'avoir lieu à Nairobi au Kenya.

Radu Codreanu

LE VI[e] SYMPOSIUM NATIONAL DE MICROSCOPIE ÉLECTRONIQUE

Les travaux de ce Symposium ont eu lieu dans les amphithéâtres de l'Institut de Pathologie et Génétique médicale « Victor Babeş », le 4 et 5 décembre 1981 à Bucarest. La séance d'ouverture fut marquée par les allocutions suivantes : des professeurs I. Moraru, directeur de

l'Institut, de la part de l'Académie des Sciences médicales et I. Teoreanu, représentant du Conseil National pour la science et la technologie, ensuite des académiciens Şt. Milcu et R. Codreanu de la part de l'Académie de la République Socialiste de Roumanie.

Parmi les communications de biologie cellulaire végétale, signalons les suivantes : Caractères ultrastructuraux des cellules de *Vitis vinifera* cultivées *in vitro* (A. Brezeanu, M. Iordan, A. Roşu, D. Mirancea) ; Etude électronomicroscopique des virus de plantes de floriculture (Al. Macovei, M. Nicolaescu) ; Ultrastructure des virus entomopathogènes utilisés contre les insectes nuisibles à l'agriculture (Z. Petre). Concernant la connaissance ultrastructurale des organismes unicellulaires, faisons mention des communications suivantes : L'ultrastructure des chromosomes du type mésocaryote (L. Gavrilă, C. Dimitriu, G. Mihăescu) ; L'apport de la microscopie électronique à élucider la systématique des Microsporidies (D. Codreanu-Bălcescu, R. Codreanu).

En rapport avec les effets pathogènes des virus, retenons les exposés suivants : Sur un virus cytomégalique murin en cultures cellulaires (S. M. Dumitrescu, I. Aderca, M. Iftimovici) ; Sur l'ultrastructure d'un virus cutané à ADN (V. Păiş, E. Păiş) ; Activités nucléaire et nucléolaire dans les lignées cellulaires infectées par l'adénovirus 3 et un cytomégalovirus (G. Mihăescu, L. Gavrilă, I. Aderca, D. Mişcalencu). Chez *Bacillus subtilis* 165, la production enzymatique glucido-protéasique est liée au mésosome (G. Mihăescu, I. Mencinicopschi, L. Gavrilă).

En pathologie cellulaire, concernant l'arthrite rhumatoïde, furent présentées des recherches sur la localisation subcellulaire de la catepsine B (E. Codorean, E. Gabrielescu, L. Buzilă), sur l'ultrastructure du macrophage activé immunologiquement (E. Gabrielescu, G. Butur, E. Codorean) et sur l'étude ultrastructurale de l'érythrophagie (N. Nicolau, E. Gabrielescu). On a également fait connaître : L'ultrastructure des cellules Langerhans, et des mélanosomes de l'épiderme ainsi que des productions intranucléaires passagères V. Păiş, E. Păiş) ; Les aspects ultrastructuraux du cortex cérébral dans l'hydrocéphalie (C. Arseni, F. Nereanţu) ; Données ultrastructurales sur la musculature dans la « multiminicore disease » (M. Alexeianu, D. Cristodorescu, C. Vasilescu, Al. Petrovici. E. F. Nedelcu) ; Examen ultrastructural de l'agression expérimentale aiguë du myocarde par la Novodrine (Isoprénaline) (D. Laky, C. Zeană, G. Filipescu, S. Socolovschi) ; Modifications ultrastructurales des hépatocytes par le pesticide Dinocton (G. Filipescu, S. Constantinescu, G. Rotaru, E. Latea) ; Etude en microscopie électronique à balayage de la membrane des macrophages sous l'action des poussières industrielles (N. Simionescu, N. Manolescu, R. Tripşa, I. Iacob, M. Bolarciuc).

Une table ronde consacrée aux débats sur les possibilités d'amélioration des techniques dans la recherche ultrastructurale fut coordonnée par P. G. Ploaie, secrétaire de la Commission de microscopie électronique.

Radu Codreanu

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 interlignes (31 lignes par page) en deux exemplaires.

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

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

Les travaux seront accompagnés d'un court résumé de 10 lignes au maximum, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages (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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