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NEW HETEROMYSINI FROM THE CORAL AREA NEAR HERON ISLAND (SE QUEENSLAND)—AUSTRALIA

BY

MIHAI BĂCESCU

In a new lot of mysidaceans collected in the coral reefs near Heron Island — Brisbane (SE Queensland — Australia) and sent to me for study by Dr. P. Davie, were massively dominating — if not exclusively — representatives of the Heteromysini tribe; only two stations (W 8721 and W 7206) included *Rhopalophthalmus brisbanensis* Hodge 1963.
Among the *Heteromysis* species already cited from there — *Heteromysis abrucei* Băcescu, *H. heronensis* Băcescu, *H. australica* Băc. & J. A. Bruce, *H. harpaxoides* Băc. & Bruce and *H. stellata* Băc. & Bruce (see **1**, **2**, **3**) — I found in the new lot *H. abrucei* (in St. W. 9376 and 9410) and *H. australica* (in St. 9393 and 9407); moreover, I also remarked the presence of *H. zeylanica* W. Tatt. (St. W 9376) and of other three new species dealt with in this paper. *Heteromysoides longiseta*; *Heteromysis tethysiana* and *H. macrophthalma*.
Like *H. abrucei* or *H. heronensis*, these new species do not seem to be compulsorily commensal with these animals, but rather inhabitants of the coral shivers.

HETEROMYSOIDES LONGISETA n.sp.

(Fig. 1 A—L)

Material. 1 subadult ♀ and 2 adult ♂♂. Heron Island. SE Queensland — Australia (St W 9400); 225 m from beach rock; 3.I.1979. Mr. Dr. P. Davie from Queensland Museum.

Diagnosis. Eyes flattened dorso-ventrally, broader than long, with a small antero-lateral corneal part; 2 setae diverging on the antero-inner corner of antennule; pleopods absolutely alike in both sexes; long plates on which the pseudo-branchial lobe is well defined only on the first pair ending in a strong phanera, with uneven relief, almost twice longer than the basis. A short wart-like ♂ lobe. With 3—4 long hairs, situated on the ventral side of A_1, just at the basis of the flagelli.

Description of the female. Slender, a bit flattened dorso-ventrally. Carapace with a slight rostral curved extension, doubled above by a slight fold; its antero-lateral corners rounded (Fig. 1 A). Abdomen, with segments weakly growing caudally (Fig. 1 B) ends in a telson armed with 7—8 spines only in the distal half. The distal spine of this growing row exceeds the insertion of the two apical spines among which the inner one is 1/3 shorter. Sinus with about 9—11 laminae; distal margins nude up to the line that would link the 3-rd pair of lateral spines (Fig. 1 C). The male shows 7 + 7 spines on telson, nearer its distal third.

Antennule (Fig. 1 A) with 2 stiff hairs divergent on its antero-inner corner and a long apophysis on the distal and outer side of the first basal segment. It is the same in the male, but the male lobe resembles a rugous

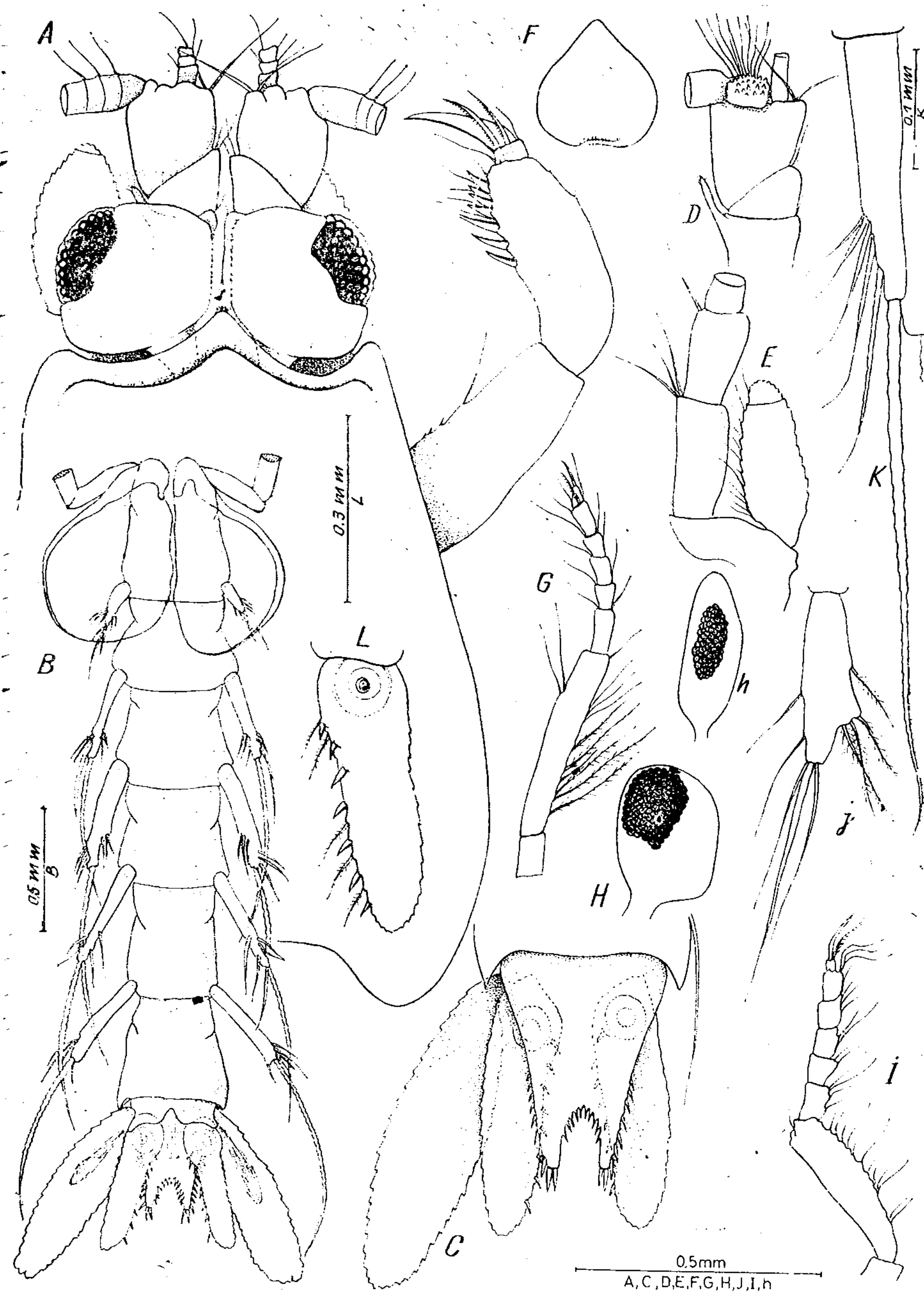

Fig. 1. — *Heteromysoides longiseta* n. sp. ♀

A, anterior side, tergal view; B, abdomen, ventral view; C, telson and uropods; D, A_1♂; E, A_2♀; F, labrum; G, peraeopod II♀; H, eye of ♀, *lateral* view; h, eye of ♂, *lateral* view; I, peraeopod IV juv. ♀; J, pleopod I♀; K, pleopod III ♀; L, endopodite of uropod.

tubercle and is provided with several long hairs between the two flagelli (Fig. 1 D).

Antenna shows an almost oval scale in ♀ (Fig. 1 E) and a bit more elongate in ♂, in both cases hardly exceeding the second segment of its basis by the apical articulation. This counts among the shortest squamae of the Heteromysini tribe. Eyes — with lateral diameter larger than the longitudinal one — strongly flattened dorso-ventrally in ♂ (Fig. 1, h) less in ♀ (Fig. 1, H), with a minute latero-anterior corneal part, more visible ventrally and especially laterally (Fig. 1, H, h). Omatidia large, buried in black-brown pigment.

Labrum triangular (Fig. 1 F); maxillae and maxillipedes common to genus *Heteromysis*.

Peraeopod I vigorous, provided with a bit dilated propodus, with a group of distally denticulate and flagellate phanerae and 3 spines on the opposite side; on the anterior edge of carpus and merus 9—11 minute spines articulated on a prominence too large for them (Fig. 1, A).

Peraeopod II, the longest, with merus longer than carpopropodus and with ± straight articulate segments which at rest stay along cephalothorax (Fig. 1, G). This one and the remainder of peraeopods show a 5-segmented carpopropodus, but their merus is shorter than carpopropodus and shows two bents, one outwards at the merus-ischium articulation and the other one down, at the tarsus-merus articulation (Fig. 1, I). Peraeopods III—VI get easily broken at the coxa-preischium articulation, between their coxae. Feeble hyposphenia; at the basis of the last pair a ± foliaceous penis, reminding of an oostegite bud; the ♀♀ show three oostegites, but it seems that they form two separate empty pockets, relying on the only adult ♀ that I studied.

The pleopods represent a remarkable morphological achievement in the Heteromysini (Fig. 1, B). Not only that they are alike in both sexes (only their distal seta is slightly shorter in ♂) but with their long basis and with their enormous distal setae with small irregular eminences (Fig. 1, K) they exceed the sides of pleonites giving an odd aspect to the abdomen and remind of the chaetae of a Polychaet. They are devoid of pseudo-branchial lobes; only pleopod I is distinct from the remainder (Fig. 1, J); it is shorter and shows a clear pseudo-branchial lobe armed with 4—5 plumose setae; as for the rest, a bunch of distal setae; the long apical seta only twice longer than the others. Usually, the large seta of the last pleopod reaches the middle of the uropodal endopodite and that of the antepenultimate one attains the statocyst (Fig. 1, B).

Uropods, especially the exopodites, with broad rami; endopodite, with a very small statolith, usually shows 8 spines all along the uropodal endopodite (Fig. 1, L) in ♀ and 7 in the ♂♂.

Size: marsupiphorous ♀ = 5.5 mm; ♂♂ = 4.2 mm

The males differ from the female only by their smaller size, the presence of the rugous protuberance on the inferior side of the extremity of antennule and by their eyes that are more flattened, sharper anteriorly and slightly excavated on the side opposite to cornea.

Holotype ♂ and one paratype (♂) in the collection of Queensland Museum, Australia (no. W 9689, resp. W 9690). Paratype ♀ in the collection of the "Gr. Antipa" Museum under no 593.

H. longiseta is the first representative of the genus found outside the N. Atlantic waters.

Remarks

It differs from *H. cotti* Calman 1932 from Lanzarote in the eyes longer than wide, in only 5 (not 6) pseudo-carpal segments; it differs from *H. spongicola* Băcescu 1968 from Cuba in the presence of 8 uropodal spines instead of 1 and in the armature of telson. From both these species, it differs in the long pleopodal plates, devoid of pseudo-branchial lobes, ending in enormous phanerae with uneven relief.

HETEROMYSIS TETHYSIANA n.sp.

(Fig. 2 A–P)

Diagnosis. Small mysids: ♂♂ = 3.2 – 3.4 mm; ♀ = 4 mm, with slightly flattened body. Peraeopod I slightly dimorphic; in exchange, it is provided with a carpo-propod poor in phanerae, but with a long merus armed with a dactyliform group of 5–6 flattened, almost superposed, flagellate spines. Antennula does not show the flagellate "spine of Olive Tattersall", but only 2 divergent spiny-hairs.

Pleopods alike in both sexes, except the IV-th pair ♂ that ends in 1 or 2 curved spiniform tubercules. No spine on uropodal endopod; telson with few phanerae, having on apex a minute spine flanked towards the exterior by the second spine 4–5 times longer.

Material. 1 marsupiphorous ♀ = 4 mm, St. W 9390; 1 adult ♂ = 3.4 mm, W 9400 and 1 adult ♂ = 3.2 mm St. W 9410, all from Heron Island, SE Queensland-Australia, for which thanks are due to Mr. P. Davie, Curator of Crustacea at Queensland Museum.

I have not other collection data except that they originate in quantitative samples (with 0.1 m² Smith-McIntyre grab).

Description ♂♀. Mysids 1.5 times broader than high; ventral face of abdomen flat; frontal side of carapace with a short rounded rostrum that hardly touches the ocular peduncle (Fig. 2 A). Eyes large; in ♂♂, larger than in ♀. Eye somehow oval in tergal view, with a postero-basal swelling and a strongly reniform corneal area seen from above (Fig. 2 A), and oval when seen laterally (Fig. 2 C), with a weak anterior mark of the passage to peduncle; omatidia large.

Thoracomeres subequal, the last a bit longer.

Telson, only a little shorter than the last thoracomere, is twice broader at the basis; naked in the proximal half, it has only 5–6 lateral spines in the terminal half, ending with 2 apical spines — the inner one minute, about 1/5 from the outer one. Sinus with 8–10 laminae all along (Fig. 2,0). In one of the males, the sides of sinus are crossed (Fig. 2, P), sinus being hardly visible. Is the tip of telson used as a prehensile organ?

Antennula (♂♀) lacks the flagellate spine that we called "Olive Tattersall's spine" characteristic of subgenus (*Olivaemysis* Băcescu (**1**); it also lacks the plumose setae and is provided only with 2 divergent spiny-hairs (Fig. 2, A). A_1 ♂ is different by the presence, on the infero-terminal side, of a ± hemispheric prominence dented on inner side and of a bunch of fine hairs longer than the segment III of antennule (Fig. 2, B).

Antenna is provided with an oval scale, 2.5 times longer than broad, but much shorter than antennule (Fig. 2, A). Labrum slightly triangular anteriorly; the morphology of the buccal parts does not deviate too much from the structural plan of genus *Heteromysis*.

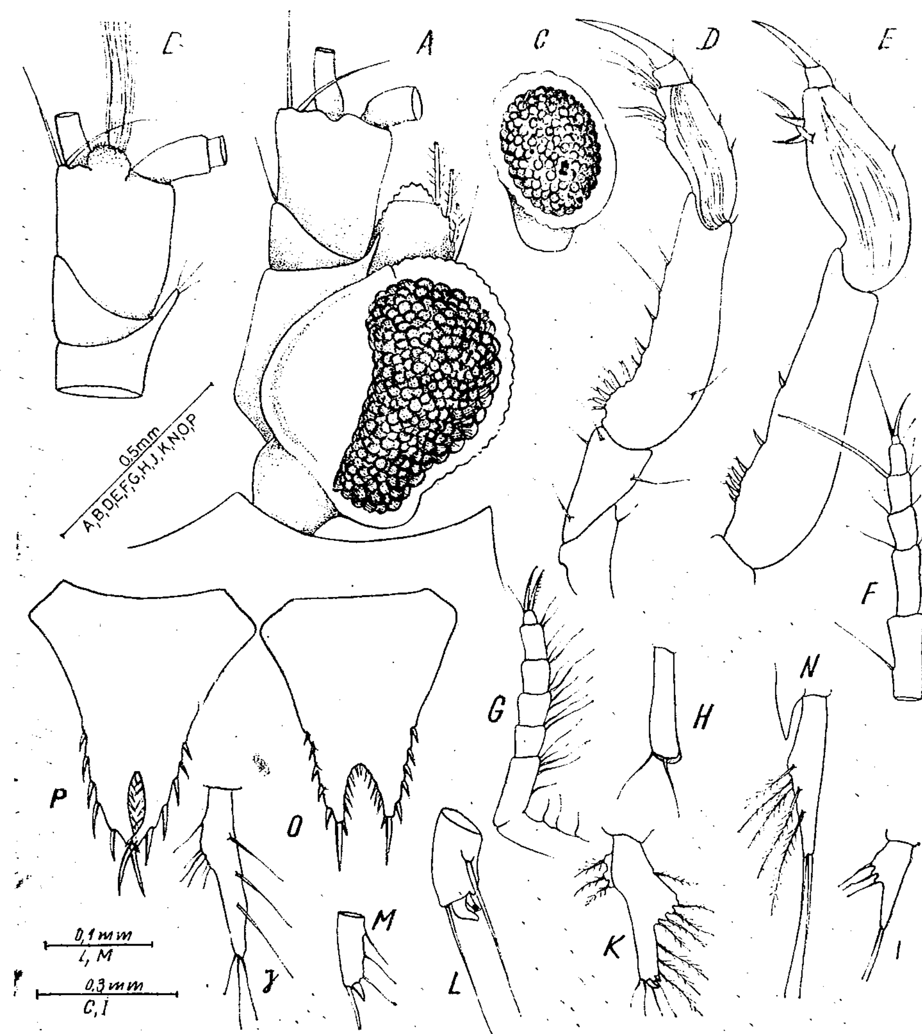

Fig. 2. — *Heteromysis tethysiana* n. sp.

A, part of anterior side of ♀, tergal view; B, A_1 ♂, lateral view; C, eye, lateral view; D peraeopod I ♀ = 4 mm; E, ditto, ♂ = 3.4 mm; F, "tarsus" of peraeopod II ♂; G, tarsus of peraeopod IV ♂; H, penis; I, pleopod I♀; J, pleopod V ♀; K, pleopod IV♂; L, its tip, magnified; M, ditto, in other ♂; N, pleopod V♂; O, telson ♀; P, telson with crossed apices (♂).

Peraeopods I and II differ from the others; the former, by a carpo-propod with few phanerae used in the movement of subchela but with a long merus armed on the inner side with a seta, 3 spines placed at intervals and a group of 4–5 dentiform, flagellate, spines in the proximal third (Fig.

2, D). It is at the level of these spines that the long dactylo-claw reaches when used as subchela.

These peraeopods show a weak dimorphism of size: carpo-propod more inflated in ♂, and both it and merus with less phanerae (compare Fig. 2, D to Fig. 2, E).

Peraeopod II with only 4 carpo-propodal segments (Fig. 2, F) with strong simple setae. The remainder of peraeopods show 6 carpo-propodal segments; it means that "tarsus" is 7-segmented (Fig. 2, G). Between the last pair of peraeopods weak hyposphenia beside 2 long ± cylindrical penes, with a slight bludgeon at the end (Fig. 2, H).

Pleopods alike in both sexes, except that of pair IV that has a well-defined pseudobranchial lobe and ends in 1 (Fig. 2, M) or 2 (Fig. 2, L) curved spines like crochets; pleopods are elongate, with straight inner side, except the first one (♂ ♀) (Fig. 2, I).

Uropods much longer than telson; endopodite with no spine at all.

Collected in association with *H. abrucei*, Băcescu, 1979

Holotype (1 ad. ♀) at the Queensland Museum under no. 9688; paratype ♂ (dissected), ibid.; no. W 9691; paratype ♂ at the "Gr. Antipa" Museum under no. 594.

Remarks

Heteromysis tethysiana, a species without uropodal spines, with an enormous outer spine at the tip of telson beside a minute inner one as well as with a particular group of spines on the proximal side of the merus of the slightly dimorphic peraeopods (probably also present in *H. dispar*, but this peraeopod lacks in Brattegard's specimens), approaches, within the vast framework of genus *Heteromysis*, only *H. digitata* W. Tatt. (whose name comes just from the above mentioned group of digitiform spines) and *H. dispar* Brattegard, 1970 [4].

The first species of this type of *Heteromysis* was described by W. Tattersal from the Suez Canal in 1927 and has not been refounds; the second — *H. dispar* — was described by Brattegard from Florida in 1970.

Our species is the third in the "digitata" group and it appears in NE Australia. This enormous area of a group of species which, like the whole subgenus *Olivaemysis*, is certainly present also in intermediary zones (I think primarily of the Bay of Bengal) — clearly indicates an old origin, from the Tethys Ocean, of these mysids; hence, the specific name that I gave. They could face the time only thanks to their commensalism or to their favourite corallicolous biotope.

The three species of the *digitata* group can be distinguished as follows:

1(2) Scale A_2 as long as A_1; the Tattersall spine present(?) and without dimorphism at pleopods (this has to be checked again, because Brattegard does not figure it) . . . . *H. dispar* (Florida)

2(1) Without Tattersall spine, with sexual dimorphism at 1 or 2 pairs of pleopods and with scale A_2 clearly shorter than A_1 . . . . **3**

3(4) With rostrum sharp, long; pleopod 3♂ with a long apical seta and pleopod 4♂ with 4 flagellate apical spines . . . . *A. digitata* W. Tatt. (Suez)

4(3) With short, rounded rostrum, with non-dimorphic pleopod III; pleopod IV with only 2 (seldom 1) curved, non-flagellate spines . . . . *H. tethysiana* mihi (Queensland — Australia)

HETEROMYSIS (OLIVAEMYSIS) MACROPHTHALMA n. sp.
(Fig. 3 A—K)

Diagnosis. Eyes big, with peduncle strongly enlarged at the basis, ending anteriorly with a spine that slightly exceeds cornea. Three carpo-propodal segments in peraeopod II and six in each of the others. Telson

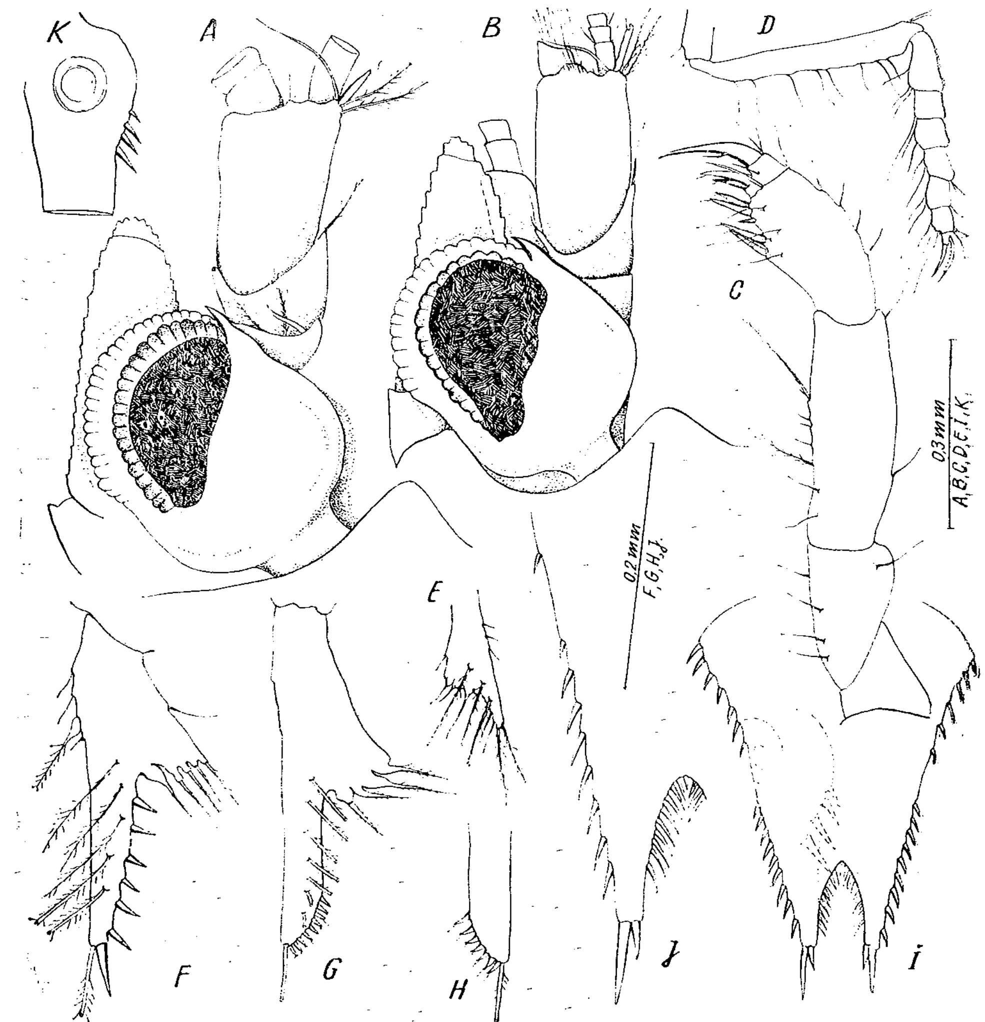

Fig. 3. — *Heteromysis (Olivaemysis) macrophthalma* n. sp. ♂♀
A, anterior side of a ♀; B, ditto, of a ♂; C, peraeopod I♂; D, peraeopod 4 ♂; E, pleopod ♀; F, pleopod II ♂; G, pleopod III♂ H, distal half of pleopod IV ♂; I, telson ♀; J, terminal side of the telson of a ♂; K, basal side of uropodal [endopod ♀.

with about 15 spines all along its sides, but with a median gap. Outer apical spines pointed, about two times longer than the inner ones. Sinus with 15—22 laminae all along. Pleopods III—IV with flagellate spines in the inner distal third and a series of strong non-flagellate spines all along the

inner distal half of pleopod II ♂ : 2–3–(4) spines at the level of the statocyst of uropod.

Material, locality. Heron Island, south-east of Queensland-Australia : 1 ad. ♂ in St. W 9390 : 200 m from beach rock, 3.I. 1972 ; 1 ad. ♀ and 1 juv. ♂ – St. W 9376, 0 m, 5.I.1979.

Description of the male. Body slightly flattened dorso-ventrally ; integument shiny ; the frontal side of carapace sketches a weak obtuse rostrum (Fig. 3 B). Antennule exceeds much the antenna and its scale ; besides the "O. Tattersall phanerae" on the A_1 tip, there are 2 long plumose setae, parallel to the flagellate spine. On its apex, between the basis of flagelli, one can observe the ♂ lobe with ventral direction, resembling the half of a sphere, armed with a rich bunch of fine hairs. The divergent seta-spine is more vigorous than the plumose setae.

Eyes large ; their peduncle enlarged at the basis marks anteriorly the beginning of cornea by a spiniform prolongation. Scale A_2 a bit longer than its peduncle, but shorter than A_1. Labrum slightly triangular, anteriorly rounded ; buccal parts and maxillipedes common to the genus.

Peraeopods I with three pairs of flagellate parallel spines, with many simple setae and 2–3 smaller spines ; the rest of phanerae as in Fig. 3 C.

Peraeopod II with only three carpo-propodal segments and pairs III – VI with six segments each (Fig. 3 D).

Pleopods I short II–V about as long as in the female (Fig. 3 E) ; those of the II pair with 7 spines : those of the third pair are armed with 9–11 flagellate spines (Fig. 3 G) occupying the caudal third of the elongate part ; the fourth pair with 7–10 subterminal spines (Fig. 3 H). Uropod provided commonly with 3 spines in the area of statocyst (Fig. 3 K).

Penis cylindrical, almost twice longer than basis + preischium of the last peraeopods.

Telson distinguished by the combination of the following characteristics : about 15–20 spines all along its sides, with a median gap, a bit larger than the space left as if one of the spines would be missing.

According to the size of the specimen, the proximal series of spines (5 – 7) decreases caudally, while the distal series (6–9) grows towards the apex. The ends of the last pair do not touch the basis of the apical spines. They are pointed, the outer one being more than twice longer (Fig. 3 J). Sinus with few laminae (15–22 in the studied specimens) which occupy the whole sinus, the caudal pairs being placed approximately on the line of the last pair of lateral spines. Endopod of uropod as in ♀ (Fig. 3 K).

Description of the female (Fig. 3 A, E, H, K)

Besides the dimorphism of the antennules and of pleopods II, III and IV, mention should be made that the female of *H. macrophthalma* is provided on peraeopod II with a carpo-propodus and a merus which are finer than in the male. As for the rest, there is no difference in outer morphology between sexes. A populational study would maybe reveal meristic differences.

Size: 4–5 mm.

Holotype: ♂ St. W 9390 in the coll. of the Queensland Museum under nr W 9692.

Paratype: ♂ j ; in the coll. of the "Grigore Antipa" Museum under no. 553.

The mysidologic biocoenosis of these stations also includes *Heteromysis abrucei* Băc. and *H. zeylanica* Tatt.

The only species of subgenus *Olivaemysis* having a median gap in the series of spines all along the sides of telson are *H. (Olivaemysis) zeylanica* W. Tatt. 1922 and *H. O. disrupta* Brattegard 1967 ; but they are armed with spines all along the uropodal endopodite, the laminae occupy only the proximal half of the sinus and the number of flagellate spines is much larger in the former than in the latter (in *H. O. disrupta*, the male is not known).

H. (Olivaemysis) macrophthalma also shows affinities to *Heteromysis pacifica* O. Tatt from the New Caledonia (telson, uropodal spines), but this one belongs to *Heteromysis* s. str. (i. e. without the "O. Tattersall phanerae" on A_1 and with enormous article III of antennule ; even if one reached the conclusion that this species, too, belongs to subgenus *Olivaemysis*, my species differs from it in the size and shape of the eyes (dilated at the basis ; a reduced corneal area) ; the distal segment A_1 of the size common to the genus) and in the spinulous pleopod II ♂.

The armature of pleopod 2 ♂ (Fig. 3 F) is less common, its distal half is armed with 7 veritable, non flagellate, pointed, spines as long as the width of the little plate supporting them. If we also find species with modified pleopod II ♂ (*H. digitata*, *H. gomezi*, *H. mayana*), this modification consists only in the presence of a long spiniform distal seta ; the appearance of a series of non-flagellate spines represents a unique morphological achievement figured among the known species, being able to characterize by itself *H. (Olivaemysis) macrophthalma* mihi.

According to the collection data (the ecological observations seem to be lacking), *H. macrophthalma* has a narrower distribution in the reef waters of Heron Island than *H. abrucei*, and *H. australica* Băcescu.

Counting the 3 present species, the number of the representatives of genus *Heteromysis* l. s. from the Australian waters becomes 11, and that of all the known species, 50 [6, 8]. Anyway, the area of the Australian coral reefs represents a sector of active adaptative radiation and speciation for this genus, like the Carribbean Sea.

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ON THE TAXONOMY AND SYNONYMY OF THE SOUTH ASIAN SPECIES OF CIRRHINUS S. STR. (*PISCES, CYPRINIDAE*)

BY

PETRU M. BĂNĂRESCU

The species of *Cirrhinus* can be assigned to two subgenera: *Cirrhinus* s. str., with 11–15 and *Henicorhynchus*, with 8 branched dorsal rays. The first subgenus includes six South Asian species: *mrigala, horai, cirrhosus,* and the doubtful *chandryi* in India, *jullieni* and *microlepis* in the Mekong and Menam basins. *Osteochilus prosemion* from Thailand is a subspecies of *C. mirgala*, *O. spilopleurus* a synonym of *C. jullieni*. Most records of *C. jullieni* actually refer to quite another species: *C. (Henicorhynchus) siamensis* (De Beaufort).

The genus *Cirrhinus* Oken, 1817 (type species: *Cyprinus cirrhosus* Bloch) ranges in the southern part of East Asia and in continental South Asia (Indian subcontinent, Burma and the basins of the Menam and the Mekong rivers), being absent from the Malayian Peninsula and the Indonesian islands. The three East Asian species were reviewed by the author in a previous paper [1]; one of them, *decorus*, is considered by Wu [11] as belonging to the genus *Labeo*. There are described six species from India (Day [2] lists five, one of which, *latius*, is actually a *Crossocheilus*; two have been described subsequently), while Smith [9] accepts for the fauna of Thailand four species of *Cirrhinus*, four of "*Tylognathus*" and one of "*Henicorhynchus*", ascribing two other species of the same group to *Crossocheilus*.

The comparison of specimens from most of the South Asian nominal species mentioned above enabled the conclusion that the present views about their classification are not correct, several of them being synonymous. The present paper represents their preliminary revision.

Material: the specimens this study is based on belong to the following collections: the Academy of Natural Sciences of Philadelphia (ANSP); Institutul de Ştiinţe Biologice, Bucureşti (ISBB); the Museum of Comparative Zoology, Cambridge, Massachusetts (MCZ); Muséum National d'Histoire Naturelle, Paris (MNHN); Stanford University, now in California Academy of Sciences, San Francisco (SU); the Natural Museum of Natural History, Washington (USNM); Zoölogisch Museum, Amsterdam (ZMA).

SYSTEMATIC ACCOUNT

Genus **Cirrhinus** Oken, 1917

The species of the genus can easily be classified in two groups: the first one includes the species with 11–15 branched dorsal rays (among these are the East Asian species and several South Asian ones, including the

generotype) and the second group (subgenus *Henicorhynchus*) includes the species having constantly 8 branched dorsal rays; no species has 9 or 10 dorsal rays.

Subgenus **Cirrhinus** Oken, 1817

(Type species: *Cyprinus cirrosus* Bloch)

1. *Cirrhinus mrigala* (Hamilton-Buchanan, 1820)

Synonyms: *Cirrh. rubripinnis* Valenciennes, 1842; *C. plumbea* Valenciennes, 1842)

Numerous available specimens, among them the holotype of *C. plumbea*, MNHN 3362, 226 mm standard length, and three syntypes of *C. rubripinnis*, MNHN 3854, 260 mm, 227.5 mm and 161 mm, the largest of them being here declared lectotype (two other specimens from the series proved to be *C. reba*)

D 4/12–13; L. lat. 41–44; Sp. br. 40–49; Circum-ped. 18 or 20. Only rostral barbels present.

A well-known, economically valuable species, ranging throughout the northern part of the Indian subcontinent and in Burma.

1 a. *Cirrhinus mrigala prosemion* (Fowler, 1934)

– Fig. 1, 2 –

Synonyms: *Osteochilus prosemion* Fowler, 1934 (Cheingmai, Menam River basin); Fowler, 1937 (Kemarat, probably Mekong basin).

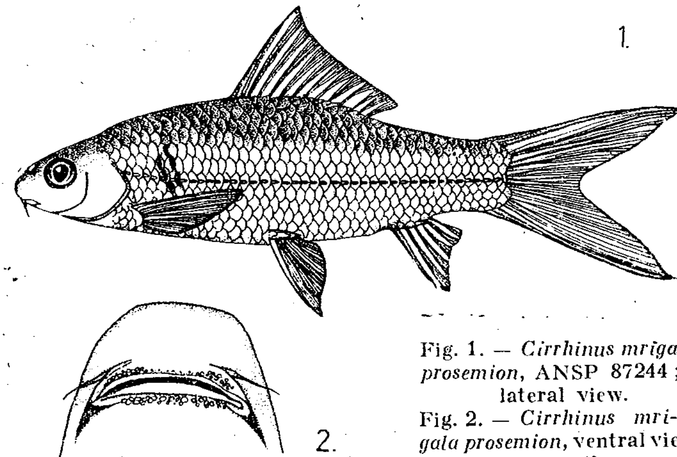

Fig. 1. — *Cirrhinus mrigala prosemion*, ANSP 87244; lateral view.
Fig. 2. — *Cirrhinus mrigala prosemion*, ventral view of mouth.

Specimens examined: paratype, ANSP 59096, Chiengmai, 89.5 mm; ANSP 87244, Kemarat, one specimen, 106 mm

D 4/11–12; L. lat. 40–41; Sp. br. 60–63; Circum-ped. 18 (on each side 4 1/2 scales above and 3 1/2 below the lateral line scale). Rostral barbel present; maxillar barbal quite rudimentary in the paratype, totally lacking in the second specimen (The original description by Fowler mentions: D 4/11–12; L. lat. 36–38; Sp. br. ± 35)

This fish, originally described as an *Osteochilus*, is actually a *Cirrhinus*, very similar to *C. mrigala*, but with slightly fewer scales and dorsal rays and with much more gill rakers. Its range includes the basins of the Menam and the Mekong rivers.

2. *Cirrhinus chandryi* Srivastava, 1968

Specimen examined: ISBB 3003, Kaveri River, Mettur reservoir at Panavadi, southern India, one specimen, 172.0 mm (received from the Zoological Survey of India, Southern Regional Station, originally determined *C. mrigala*.

The status of this species is doubtful. Its original description [10], after specimens from Gorakhpur, Uttar Pradesh, Ganges basin, is quite short, mentioning D 3/12, L. lat. 43, both pairs of barbels present, body proportions practically the same as in *C. mrigala*. The only difference between the two species concerns the barbels: *mrigala* has only rostral barbels, *chandryi* also the maxillary ones.

The available specimen, here tentatively identified as *C. chandryi* has: D 4/12; L. lat–43–43; Circumped. 18; Sp. br. ; maxillary barbel well developed on the left, rudimentary on the right side.

Since the presence of maxillary barbels is subject to individual variability within some species of *Cirrhinus*, subgenus *Henicorhynchus*, it is questionale whether *chandryi* is actually a valid species or is based on an intrapopulational morph of *C. mrigala*.

Both *mrigala* and *chandryi* were recorded up to now only in northern India. The specimen here, reported from Kaveri, may belong to an introduced stock.

3. *Cirrhinus horai* Lakshmanan, 1966

No specimen available.

D 4/15–16; L. lat. 44–46; only rostral barbels present; a conspicuous streak of orange colour along the longest ray of the lower lobe of the caudal fin.

Endemic to Godavari River basin (central-eastern India, Bengal Gulf drainage).

The species differs from *C. cirrhosa* in the number of dorsal rays

4. *Cirrhinus cirrhosus* (Bloch, 1785)

Synonyms: *Dangila leschaunaulti* Valenciennes, 1843; *Cirrhina blochii* Valenciennes, 1843; *Cirrhinus cuvieri* Jerdon, 1849.

Specimens examined: USNM 165118, Kaveri River, one specimen, 95 mm; holotype of *D. leschaunaulti*, MNHN 3852, Pondichery, south-eastern India, 184 mm.

D 4/13–14; L. lat. 43–46; Circum-ped. 20 or 22 ($4^1/$ or $5^1/2$ scales above and $4^1/2$ below the lateral line scale); Sp. br. / ± 60. Both pairs of barbels well developed.

Confined to the basins of the rivers from South India flowing into the Gulf of Bengal, from Godavari to Kaveri.

5. *Cirrhinus jullieni* 1878

Synonyms: *Osteochilus spilopleurus* Fowler, 1935 (Srisawat); non *Cirrhina jullieni* Sauvage, 1881, *Cirrhinus jullieni* Fowler, 1934, 1935, 1937, Smith, 1945.

Specimens examined: Syntypes of *C. jullieni*, MNHN 8586, lower Mekong River in Vietnam (former Cochinchina), six specimens, 89.0–113.0 mm; the largest is here declared lectotype; it retained the original

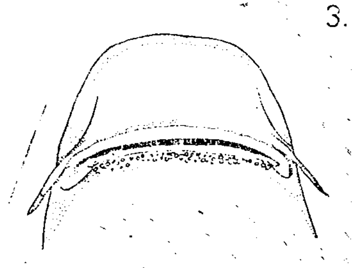

Fig. 3. — *Cirrhinus jullieni*, ANSP 60810; ventral view of mouth.

catalogue number, the five others becoming MNHN; ZMA 112894, Mekong River in Thailanda, determined by H. M. Smith as *C. chinensis*; ANSP 60810, Srisawat, basin of the Mekong, two specimens, 133.0 and 120.0 mm standard length to last vertebra (139.0 and 123.0 mm to end of scales), determined *Osteochilus spilopleura* by Fowler.

D 4/13–15; A 2/5; L. lat. 36–38 (39); Sp. br. 53–60; Circ-ped. 18.

Only rostral barbel present. Body depth 28.8–34.6% of standard length (to last scale); least depth 11.9–13.7%; caudal peduncle 16.3–18.5%; head 20.4–23.7%; snout 5.4–7.5%; eye diameter 6.6–9.4%; snout 26.6–33.0% of head; eye 30.0–37–6% of head and 61.5–84,0% of interorbital width.

Much confusion occurred about the status of this species. Sauvage's 1878 original description [7] is quite short and without illustrations; it was based on six syntypes. Three years later, Sauvage [8] gave a more complete and illustrated description of "*C. jullieni*", based on new specimens which actually belonged to quite another species, with eight branched dorsal species. All subsequent records of "*C. jullieni*" from Cambodia, southern Vietnam and Thailand by various authors, including Fowler [4, 5, 6] and Smith [9] refer to the second species, that proved to be quite common in the basins of the Mekong and the Menam rivers. Fang [3], was the only author who realized this confusion; he examined Sauvage's 1878 syntypes and proposed the new name *C. sauvagei* for the species misidentified by Sauvage [8] with his own *C. jullieni*. Actually, Sauvage's 1881 species was already described as *Tylognathus siamensis* De Beaufort, 1927; further synonyms are: *T. brunneus* Fowler, 1934, *T. entnema* Fowler, 1934, *Cirrhinus marginipinnis* Fowler, 1937 *Hemicorhynchus lobatus* Smith 1945, and perhaps *Tylognathus caudimaculatus* Fowler, 1934. This species, the right name of which is *Cirrhinus (Henicorhynchus) siamensis* (De Beaufort, 1927) will be dealt with in a subsequent paper.

Concerning the true *C. jullieni*, Fang expressed the opinion that this is a synonym of the East Asian *C. chinensis* Günther. Actually, *C. chinensis* has fewer dorsal rays, more scales (D 4/11–13, L. lat. 39–41, rarely 42) and the upper lip is papillose (*1*, Fig. 1) while in *C. jullieni* it is smooth (Fig. 3). The last named is specifically distinct.

According to its description, *Osteochilus spilopleurus* Fowler is the same species. The author could not examine the holotype and single specimen after which this nominal species was described, but two specimens from the type locality, Srisawat, ANSP 60810, determined by Fowler as *spilopleurus*, proved to be *C. jullieni*.

6. *Cirrhinus microlepis* Sauvage, 1878

Synonyms: *Cirrhina microlepis* Sauvage; *Cirrhina aurata* Sauvage 1878

Specimens examined: Holotype, MNHN 9648, Mekong R.; syntypes of C. aurata, MNHN 3849, Pnom-Penh, Mekong basin, two specs., 124.0 and 118.5 mm; the smallest specimen is declared lectotype; it retained the original number; the paralectotype becoming B. 2835; USNM 107998, Menam River, two specs., 125.2 and 149 mm; ZMA 112895, Meklong River, tributary to Menam, one spec., 146 mm; ANSP 61789-90, Menam River at Bangkok, two specs., 165 and 178 mm; USNM 28528, Mekong River in Laos, one spec., 129 mm.

D 4/11–13; L. lat. 57–59; Sp. br. 58–85; Circ.-ped. 26–30.

This species, the range of which includes the basins of the Mekong and the Menam Rivers in Thailand and adjacent countries, is the most differentiated within the subgenus, having the highest number of scales, the mouth subterminal (as against inferior) and rather transversal (not horse-shoe shaped) and both pairs of barbels lacking.

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LES CRITÈRES MORPHOLOGIQUES DE L'ESPÈCE NE SE RAMÈNENT PAS AU CONCEPT TYPOLOGIQUE

PAR

EUGEN V. NICULESCU

The author shows that the typological species concept is outdated and that there is no connection between morphology and the typological concept. All zoologists today are morphologists, including those who have adopted the biological concept of the species. The relation established between morphology and the typological concept is wrong, in fact it does not even exist. Every taxonomic study is based on morphology, the critics themselves of the morphological criterion are refuting it only theoretically, making exclusive use of it in all their taxonomic works.

Maints biologistes et zoologistes affirment que parmi les concepts actuels de l'espèce se trouve aussi le concept typologique. C'est une affirmation répétée sans vérification et sans aucun examen critique.

Notre étonnement est explicable puisque ces biologistes affirment d'une part que « le concept typologique de l'espèce s'appuie sur la théorie de Linné concernant la fixité et l'immuabilité des espèces » [3] et d'autre part ils considèrent que ce concept est actuel !

Comment peut être actuel un concept fixiste et créationniste ! Y a-t-il, actuellement, des zoologistes typologues-idéalistes[1] c'est-à-dire fixistes et créationnistes ? Certainement pas, soit qu'ils délimitent, identifient et établissent les espèces exclusivement d'après les caractères morphologiques, soit d'après un « ensemble de caractères ».

Une autre affirmation de ces biologistes est que « le concept typologique de l'espèce correspond à l'espèce morphologique ou morpho-species » [3]. Cette idée de Cain se répète sans cesse et a abouti à tort au discrédit du critère morphologique. Il est tout à fait dépourvu de sens le rapprochement de la notion de morphologie du concept typologique de l'espèce. On affirme que « conformément à la conception typologique, pour caractériser l'espèce il faut nous baser sur les caractères morphologiques ». Mais qu'est-que ça l'espèce morphologique ? Selon A. Cain(2) l'espèce morphologique est l'espèce *qui a été établie sur la seule base des données morphologiques*. Nous nous demandons : si la même espèce aurait pu être établie uniquement d'après les caractères biologiques (l'isolement reproductif) ou écologiques serait-elle biologique ou écologique ?

Le non-sens de cette notion résulte aussi du fait que toutes les espèces aujourd'hui connues (et bien entendu aussi les espèces fossiles) ont été et sont encore établies exclusivement d'après les caractères morphologiques.

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[1] C'est l'expression de S. G. Kiriakoff ; mais ce lépidoptériste s'est rendu compte, enfin, qu'elle ne peut être adressée aux morphologistes contemporains (lui-même morphologiste) et c'est pourquoi il l'a remplacée [4] par « taxonomistes-conventionnels ».

Poursuivons en analysant la conception de A. Cain [2] qui a caractérisé l'espèce morphologique comme suit :

1. Elle est statique et n'inclut pas de modifications dans le temps et l'espace.
2. Elle est monotypique et contient seulement un groupe d'individus qui se rapprochent beaucoup d'un seul type de variation.
3. Elle représente la plus inférieure catégorie taxonomique.
4. Elle se détermine exclusivement sur la base des caractères morphologiques.
5. Chaque espèce se distingue presque toujours clairement de ses parents les plus proches.

Dans un travail antérieur[8] nous avons analysé en détail l'espèce « morphologique » de Cain. Nous précisons encore une fois que l'espèce morphologique de Cain n'est autre chose que l'espèce immuable de Linné. Mais l'espèce polytypique? Celle-ci ne peut être « morphologique » sensu Cain puisqu'elle n'est pas statique et comprend des modifications dans l'espace c'est-à-dire elle est variable (variation géographique). Or, les expèces polytypiques constituent la majorité écrasante du règne animal !

D'autre part Cain considère le critère morphologique déficient pour le motif que l'espèce typologique de Linné était établie seulement d'après des caractères morphologiques. Mais Linné a fauté non parce qu'il établissait des espèces uniquement d'après des caractères morphologiques mais pour le motif qu'il a ignoré le dimorphisme saisonnier *(Araschnia levana* et *prorsa)*, le dimorphisme sexuel (l'oiseau le Colvert) et surtout la grande variation individuelle et géographique ce qui l'a déterminé à admettre la fixité de l'espèce. Ainsi s'est engendré l'idée erronée de critiquer le critère morphologique[1].

Il semble que nos idées ont convaincu E. Mayr en ce qui concerne le fait que l'espèce est aussi une unité morphologique puisque dans un travail récent [5] il dit : « L'isolement reproductif, les différences écologiques et la distinction morphologique sont les trois propriétés les plus caractéristiques de l'espèce ». Donc, à l'encontre de ses idées antérieures, Mayr admet maintenant que l'espèce n'est pas seulement une unité reproductive et écologique, mais aussi une unité morphologique, en confirmant, ainsi l'une de nos idées antérieures [7] : « Les espèces forment des communautés

[1] Parmi d'autres « arguments » apportés par les biologistes à l'appui de la « déficience » du critère morphologique, mentionnons ici le « célèbre » argument de Cain [2] qui constate une énorme différence entre un papillon et sa chenille ce qui prouverait, dit Cain, la faiblesse du critère morphologique ! Mais aussi dans les travaux récents [3] on interprète faussement les différences morphologiques.
Ainsi on dit que « si nous nous basions seulement sur des différences morphologiques nous devrions classer dans des espèces différentes les individus qui présentent les variations mentionnées ce qui constituerait une erreur ». D'ici, la conclusion erronée de la déficience du critère morphologique ! Certainement, si nous considérons comme caractères spécifiques quelques *simples variations individuelles* dans l'habitus, nous commettons une grande erreur taxonomique comme c'est arrivé, jadis, dans la Lépidoptérologie et Malacologie. Mais les caractères morphologiques ne signifient pas seulement des variations individuelles et ni seulement les caractères de l'habitus. Ils sont très nombreux ; nous les avons analysés et avons montré, pour chacun en particulier, leur valeur pour la taxonomie des Lépidoptères [9]. Ce n'est pas le critère morphologique qui est déficient mais la méthode de ces entomologistes incompétents qui n'ontp as su faire une discrimination rigoureuse entre les caractères réellement spécifiques et les simples variations individuelles.

reproductives isolées par des barrières sexuelles des autres communautés reproductives ». Cette « petite » définition de l'espèce illustre bien l'idée ci-dessus, à savoir que l'espèce est en même temps biologique et morphologique. Le corollaire de cette idée est que la conception que nous devons avoir de l'espèce n'est pas une conception biologique, mais une conception morpho-biologique. Mais les disciples sont toujours plus orthodoxes que le maître, car l'un de ceux-ci dit : « le critère morphologique ne peut constituer une base scientifique pour la caractéristique des espèces. Malheureusement il y a encore des zoologistes et surtout des botanistes qui se basent exclusivement sur les caractères morphologiques dans la classification des organismes » [3]. Nous affirmons qu'actuellement *tous* les zoologistes utilisent en exclusivité des caractères morphologiques à tous les niveaux taxonomiques. Cela ne signifie pas que ces zoologistes sont fixistes, qu'ils n'acceptent la notion d'espèce polytypique, qu'ils ne reconnaissent pas le phénomène réel de l'isolement reproductif. Cela signifie, par contre, que les zoologistes utilisent le critère de l'espèce le meilleur, que l'espèce est aussi une unité morphologique et que d'autres critères sont soit trompeurs (le critère écologique et le critère cytogénétique) soit inapplicables pour l'établissement ou l'identification des espèces (le critère de l'isolement reproductif). Nous avons montré dans plusieurs travaux [9, 10, 11, 12] qu'il y a de nombreux critères de l'espèce mais le meilleur, le plus pratique et le plus accesible est le critère morphologique — au moins chez les Insectes.

Pour conclure, nous soulignons de nouveau que la caractéristique de l'espèce selon les critères morphologiques n'a aucun rapport avec la conception typologique — fixiste. Est erronée l'opinion que « le diagnostique de l'espèce basée seulement sur des caractères morphologiques aboutit d'habitude à des résultats faux ». Il est bien connu par tous les taxonomistes que toutes les diagnoses et toutes les clés dichotomiques à tous les niveaux taxonomoques sont élaborées sur la seulement base des caractères morphologiques. Les critères morphologiques — bien choisis et justement interprétés — constituent la base d'une taxonomie scientifique, d'une classification naturelle. D'ailleurs le rejet du critère morphologique se pratique toujours théoriquement (dans quel but?) car dans la phylogénie, taxonomie et classification cela n'est pas possible.

La pensée typologique doit être réfutée non pas pour le motif que dans les diagnoses des espèces on adopte pour critère de base les caractères morphologiques, mais parce qu'elle est fixiste et créationniste, tandis que la « pensée morphologique » est évolutionniste [12]. Sur quels critères se sont basés les biologistes qui ont élaboré la conception de l'espèce polytypique? N'est-ce pas sur les critères morphologiques? Seule la morphologie a établi que les races géographiques diffèrent morphologiquement l'une de l'autre et l'ensemble des races d'une espèce constituent l'espèce polytypique.

Où est ici la pensée typologique? Mais cette conquête de la morphologie a été assumée par les biologistes adeptes du concept biologique et — chose étrange — ce sont précisément ceux qui critiquent le critère morphologique ! C'est regrettable que les idées excessives de Cain, Kiriakoff, Heydémann, etc. ont conquis de nombreux zoologistes et biologistes contemporains qui les ont adoptées entièrement sans aucun discernement. Mais il y a aussi des biologistes qui ont considéré avec lucidité le problème de

l'espèce. Ainsi N. Botnariuc [1] ne dit nulle part que « le critère morphologique ne peut constituer une base scientifique pour caractériser une espèce ». Il montre clairement que « la conception typologique de l'espèce a été élaborée dans la période Ray-Linné et dérive directement du créationnisme ». Enfin soulignons la forte contradiction de la pensée de Cain — celle-ci aussi assimilée par les biologistes contemporains. Dans sa caractérisation de l'espèce Cain dit que les espcèes se distinguent clairement de leurs proches parents. Mais comment se distinguent les espèces entre elles sinon sur des bases morphologiques !? Et toutefois le critère morphologique est considéré déficient !

Il faut admettre que le critère morphologique est celui qui définit le mieux l'espèce, qu'il est un critère de premier ordre et *l'acceptation des caractères morphologiques pour critère de base pour la diagnose des espèces n'a absolument aucun rapport avec la conception typologique; la morphologie aboutit aux conclusions du plus pur évolutionnisme* [12].

On peut dire donc qu'actuellement il n'y a que deux concepts sur l'espèce :

1. Le concept biologique (Mayr).
2. Le concept morpho-biologique (Niculescu).

Le concept typologique est du domaine du passé et il n'a plus qu'un intérêt historique.

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LES ASPECTS DE L'INTOXICATION PAR MERCURE DANS LE FOIE DE *CYPRINUS CARPIO*

PAR

MARIA TEODORESCU, VIORICA MANOLACHE et SIMONA MARCOCI

Cronic effects of the cumulation of different doses of Hg were studied at the following intervals : 2 weeks — 18 months. We have remarked some phenomena in the liver of *Cyprinus carpio* : progressive metabolic degradation, temporary tissue regenerative reaction, quick and strong alteration in many liver cells. The onset of necrosis hindered the liver to function at its whole capacity.

La pollution chimique représente la source de plusieurs formes d'intoxications à effets au niveau morphofonctionnel, présentant parfois des répercussions génétiques, mutagènes ou cancérigènes. Le tissu hépatique accuse la plus grande sensibilité envers la toxicité des résidus chimiques et, en même temps, la plus grande capacité de leur détoxification. Les réactions chimiques de détoxification nécessitent une consommation d'énergie fournie par le méthabolisme glycogénétique [1, 2, 8]. Les fonctions antitoxique et glycogénique des hépatocytes apparaissent synchroniquement dans l'embryogenèse.

Le présent travail constitue une recherche de toxicologie expérimentale qui teste, à divers intervalles et doses, la sensibilité des hépatocytes au mercure. On sait que les résidus mercuriques influencent le métabolisme des cellules et bloquent la prolifération cellulaire [10].

MATÉRIEL ET MODE DE TRAVAIL

Les effets toxiques du mercure ont été étudiés en doses de 1,5 et 10 microgrammes. Le traitement histologique du foie de *Cyprinus carpio* a été effectué aux suivants intervalles après l'intoxication ; 2 semaines, 1, 2, 3, 4, 5, 8 mois, pour la dose de 1 microgramme ; aux mêmes intervalles et à un an pour la dose de 5 microgrammes ; à 1,5 mois, 6 mois, un an et un an et demi pour la dose de 10 microgrammes.

RÉSULTATS

INFLUENCE DE LA DOSE DE 1 MICROGRAMME

Après 2 semaines de traitement l'aspect morphologique présente une baisse brusque du métabolisme des hépatocytes, manifestée par la diminution de la taille et de la quantité de cytoplasme, la densification du suc nucléaire, l'agrandissement des nucléoles. Il est à signaler également la tendance d'obstruction des vaisseaux sanguins et des canalicules biliaires.

Après 1 mois de traitement, dans la majorité des cellules s'accumulent graduellement des produits intracytoplasmatiques de réserve qui hypertrophient les cellules et compriment le noyau ; les capillaires sinusoïdes restent obstruées (Planche I, fig. 1).

Après 2 mois de traitement, les altérations cytoplasmatiques s'associent aux altérations nucléaires, suivies immédiatement par l'autolyse et la désintégration de nombreuses cellules.

Après 3 et 4 mois de traitement, dans le champ histologique abondent de nombreuses cellules hépatiques de petite taille. Bien qu'elles soient petites, ces cellules paraissent résistantes à l'action du toxique. Elles constituent probablement une réserve cellulaire de jeunes hépatocytes, utilisés au moment où la majorité des cellules hépatiques se sont ressenties après l'intoxication, accusant des altérations profondes semblables à celles décrites ci-dessus.

Après 5 mois de traitement, les cellules régénératives, ainsi que le reste des cellules hépatiques présentent les mêmes manifestations de dégénérescence. Arrivés dans la phase finale de l'hypertrophie, de nombreux hépatocytes se désintègrent, à leur place apparaissant des espaces anormaux. L'altération la plus fréquente est celle par dégénérescence adipeuse (Planche I, fig. 2).

Après 8 mois de traitement, les altérations persistent, les rapports intercellulaires sont affectés, les plasmalemmes deviennent de plus en plus fragiles et la structure typique du tissu hépatique n'est plus reconnaissable (Planche I, fig. 3). L'accumulation d'inclusions amorphes intracytoplasmatiques des hépatocytes, des espaces intercellulaires et même des canalicules biliaires est plus fréquente qu'elle était jusqu'à présent. Ces inclusions représentent une forme d'accumulation des résidus mercuriques au niveau tissulaire qui apparaît au moment où la capacité de détoxification du foie a été dépassée.

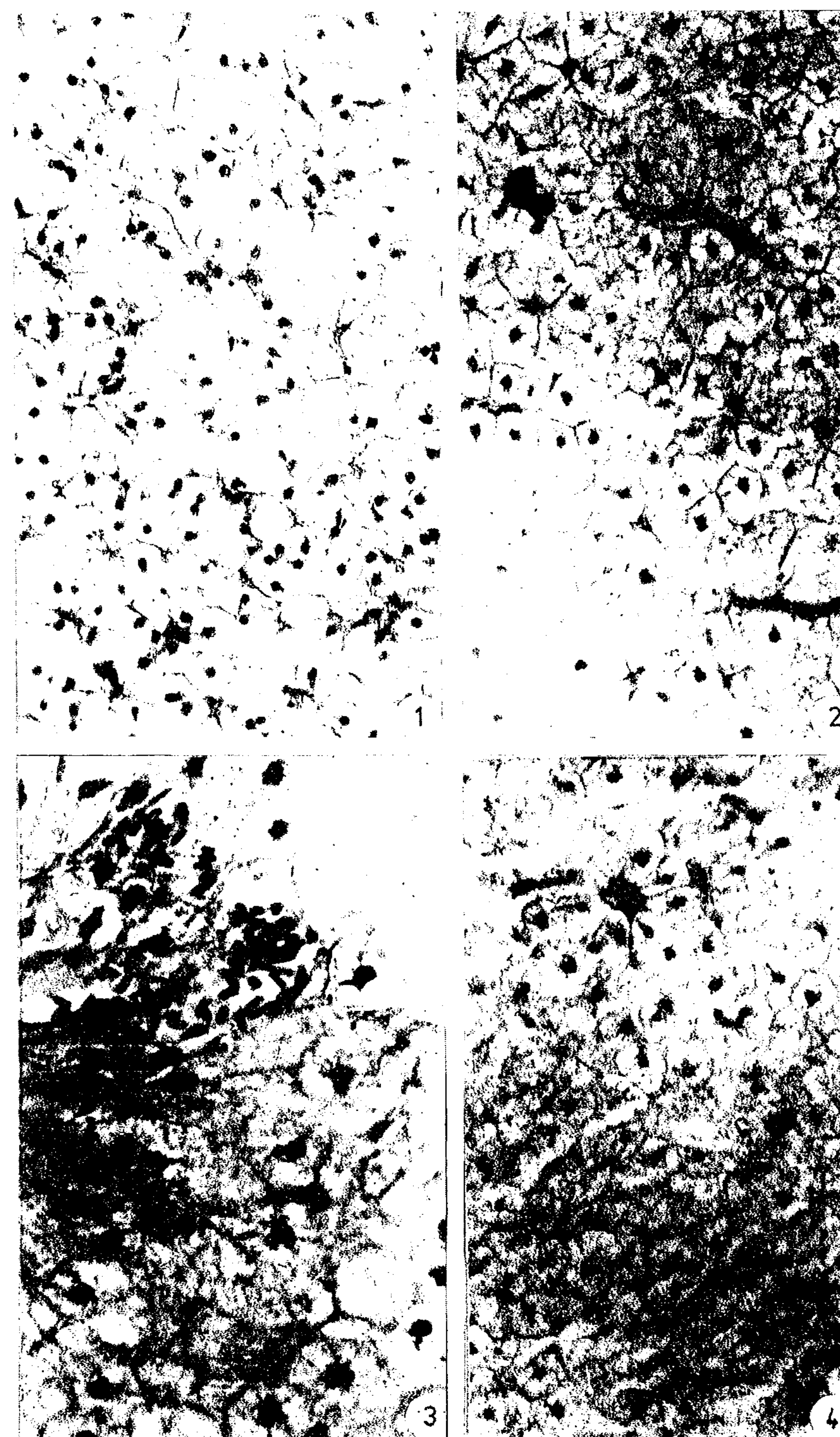
On constate que la dose de 1 microgramme se comporte comme une dose de « choc », provoquant des altérations atteignant dans de nombreuses cellules l'intensité des fortes doses de mercure. Nous avons signalé que cet aspect est plus réduit dans le cas de certains territoires où les cellules régénératives remplacent les cellules détruites. Cette étape compensatrice est toutefois temporaire étant donné que le mercure, qui continue à s'accumuler, détruira aussi le lot de cellules régénératives.

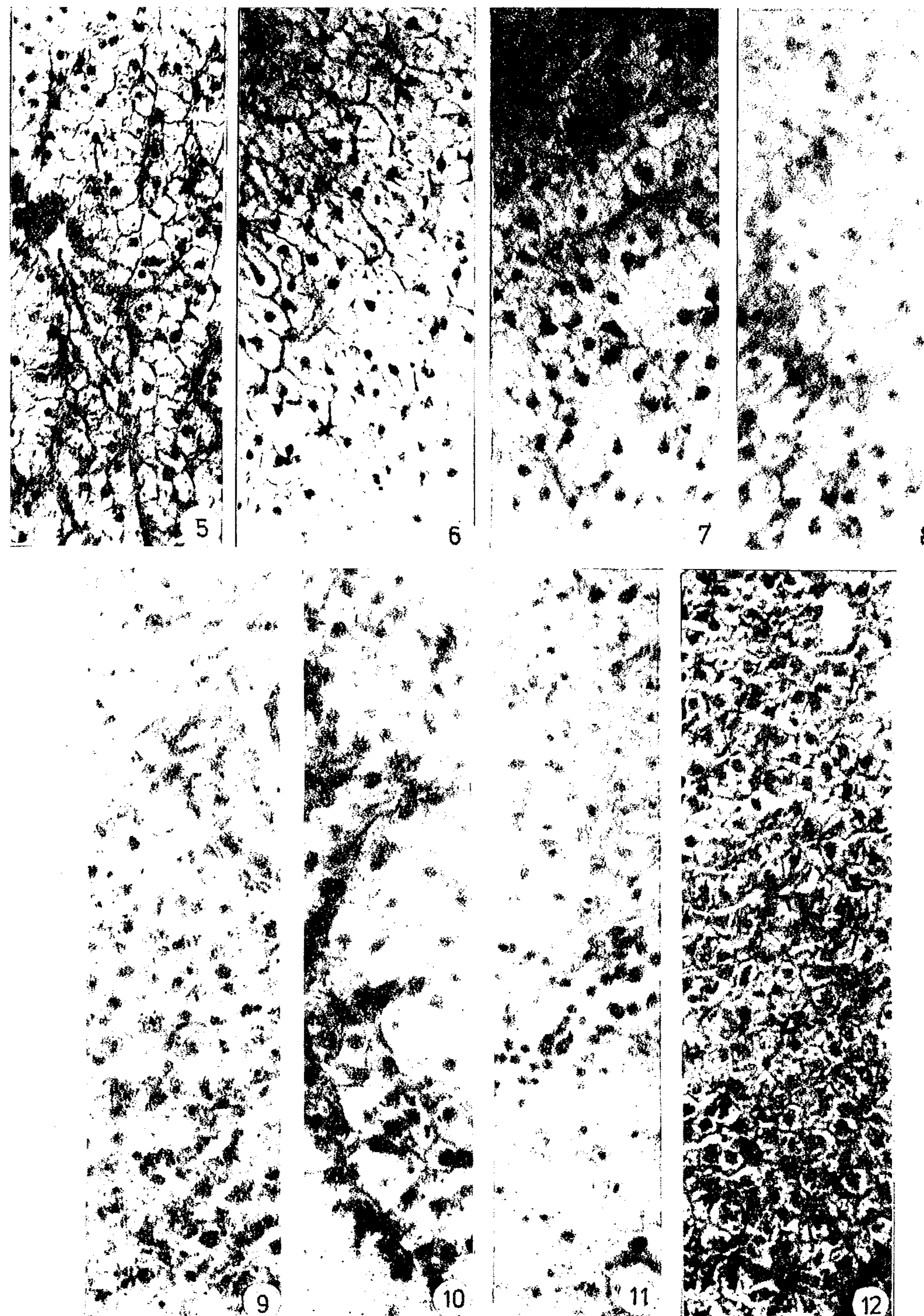
INFLUENCE DE LA DOSE DE 5 MICROGRAMMES

Après 2 semaines de traitement, cette dose agit elle aussi comme une dose de choc, déterminant de profonds troubles dans la morphofonctionnalité de la cellule hépatique. L'accumulation anormale, en excès, des produits de réserve métaboliques et l'alourdissement de leur transit vers la voie sanguine et biliaire caractérisent cette expérience. La vacuolyse intracytoplasmatique et l'hypertrophie cellulaire deviennent les principales

PLANCHE I
Foie de Cyprinus carpio sous l'action de la dose de 1 µg Hg. Fig. 1. — à une mois de traitement ; Fig. 2. — à 5 mois de traitement ; Fig. 3. — à 8 mois de traitement
Foie de *Cyprinus carpio* sous l'action de la dose de 5 µg Hg ; Fig. 4.—à 2 semaines de traitement.

PLANCHE I





causes de la désintégration de nombreuses cellules et de l'effacement de la configuration des tubes hépatiques (Planche I, fig. 4).

Après 1 mois de traitement, l'aspect des hépatocytes reste hypertrophique, mais parfois l'autolyse apparaît également en commençant par la pycnose des noyaux. L'hypertrophie s'installe graduellement ; dans certaines cellules l'autolyse commence même avant hypertrophie. Les capillaires sanguins se maintiennent comprimés dans nombreux territoires hypertrophiques (Planche II, fig. 5).

Après 2 mois de traitement, prédominent les cellules de petite taille et aux noyaux pareils que chez le témoin ; elles ressemblent aux cellules régénératives.

Après 3 et 4 mois de traitement., des cellules régénératives persistent parmi les cellules qui ressentent en majorité la toxicité et passent par toutes les étapes de l'hypertrophie (Planche II, fig. 6, 7).

Après 5 mois de traitement, le tableau histologique présente aussi bien des cellules altérées que des cellules régénératives.

Après 8 mois de traitement, on peut observer des zones de densifications et des zones hypertrophyques ; toutefois les zones régénératives, densifiées, entrent en dégénérescence, les noyaux deviennent pycnotiques, disparaissant même dans certaines cellules (Planche II, fig. 8).

Après 1 an de traitement, l'image d'ensemble révèle que le tissu hépatique dispose de moins de cellules capables de se régénérer. Les tubes hépatiques perdent la configuration normale et contiennent des cellules fortement lésées, à noyaux polymorphes et nucléoles hypertrophiques ; les rapports intercellulaires sont perturbés (Planche II, fig. 9).

En totalisant les résultats de l'effet Hg à 5 microgrammes, on constate que pendant toutes les périodes utilisées l'agent toxique trouble l'activité métabolique des hépatocytes. Néanmoins, entre 3—5 mois, l'existence des cellules hépatiques potentielles atteste une tendance de reconstitution du tissu, mais ce phénomène est passager. Le déficit de vascularisation aggrave le processus de dégradation des hépatocytes.

INFLUENCE DES DOSES DE 10 MICROGRAMMES DE MERCURE

Après 1,5 mois de traitement, la réaction de diminution des cellules apparaît uniforme dans la masse de tissu hépatique.

Après 6 mois de traitement, les résultats sont semblables à ceux enregistrés après 8 mois de traitement à 5 microgrammes Hg. L'hypertrophie englobe toute la masse du tissu hépatique et les cellules régénératives entrent en dégénérescence. A la place des cellules détruites apparaissent des espaces (Planche II, fig. 10).

Après 12 mois de traitement, au niveau histologique les résultats sont similaires à ceux obtenus après le même intervalle avec la dose de 5 microgrammes. De nombreuses cellules entrent en autolyse avant même de passer par la phase d'hypertrophie (planche II, fig. 11).

PLANCHE II

Fig. 5. — à 1 mois de traitement ; Fig. 6. — à 3 mois de tratement ; Fig. 4. — à4. mois de traitement ; Fig. 8. — à 8 mois de traitement ; Fig. 9. — à 1 année de traitement.

Foie de *Cyprinus carpio* sous l'action de la dose de 10 µg Hg.

Fig. 10.—à 6 mois de traitement ; Fig. 11. — à 12 mois de traitement ; Fig. 12. — à 18 mois de traitement.

Après 18 mois de traitement, tout comme dans l'expérience précédente, les cellules entrent en altérations avant l'installation de l'hypertrophie, altération généralisée sur la majorité des noyaux des hépatocytes (Planche II, fig. 12).

En totalisant les résultats déterminés par la dose de 10 microgrammes, on peut affirmer que le tissu hépatique s'altère brusquement dès le premier essai expérimental. Les altérations persistent pendant tout l'intervalle des expériences et se généralisent en final.

DISCUSSIONS ET CONCLUSIONS

L'action des résidus mercuriques au niveau tissulaire a préoccupé de nombreux chercheurs [1 — 5, 7, 8, 10]. Malgré sa capacité de détoxification caractéristique, le tissu hépatique possède une sensibilité qui peut aisement être mise en évidence par des expériences avec diverses toxines [6]. Le tissu hépatique répond aux noxes par : suffusions hémorragiques, infiltrations leucocytaires, dégénérescence grasse, et même necrose [9, 11, 12]. En testant l'effet des résidus mercuriques dans le foie de carpe, nous avons constaté que les dégénérescences d'ordre métabolique s'installent immédiatement, persistent longtemps, même quand la concentration en Hg est faible (1 et 5 microgrammes). Si la dose s'accroît (10 μg), l'activité des cellules hépatiques est inhibée, dès le début (1,5 mois), phénomène qui se maintient durant toute la période de l'expérience jusqu'à 18 mois, période pendant laquelle de nombreuses cellules, mêmes celles régénératives, entrent en dégénérescence très brutale, qui affecte fortement leur structure.

La réaction régénérative signalée dans le tissu hépatique [6], constatée également par nous dans le cas de l'intoxication par mercure, paraît être un phénomène de durée brève, insuffisant par rapport à l'effet cumulatif du toxique. Dans la phase finale de nos expériences, le foie ne fonctionne plus avec toute sa capacité parce que de nombreuses cellules entrent en nécrose et se désintègrent.

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REGULATION OF PARATHYROID SECRETION BY CALCIUM IN THE LIZARD *CALOTES VERSICOLOR*

BY

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Although control of parathyroid secretion in mammals has been studied in detail, little is known about the other submammalian vertebrates. Particularly reptiles are neglected in this respect [1, 2]. In our previous study we have made an attempt to correlate the activity of the parathyroid gland of the lizard with the circulatory blood calcium level [3]. In the present study, a more valuable method, such as tissue culture, is employed to get more authentic information regarding the control of parathyroid secretion.

MATERIAL AND METHOD

The eggs of the lizard *Calotes versicolor* were collected from uteri of the gravid females and incubated in a chamber at room temperature (23° to 32° C). The eggs were laid on a clean cotton bed flooded with distilled water. The stages of the development of the embryo referred to in this study are according to Muthukkarappan *et al.* [6].

The 35 th-stage embryos were obtained after removing the egg shell and maintained in the cultured media containing different concentration of calcium ions (Shinda, unpublished). The parathyroid glands from the respective material were transplanted at this stage of the embryo and maintained in a BOD/COD incubator for three days. After completion of the experiment, the parathyroid glands were fixed in Bouin's fluid for 24 hours. Six-μm-thick paraffin sections were stained with haematoxylin and eosin. Cells per macroscopic area and the nuclear cytoplasmic ratio (N/C Ratio) were determined as described previously (4). For numerical data the Student *t* test was used.

OBSERVATIONS

The histological features of the parathyroid gland of the lizard *Calotes versicolor* are more or less similar to other lizards described previously [5].

The parathyroid gland maintained in low calcium ion media shows hyperactivity. Cells per microscopic area and the N/C Ratio of the parathyroid gland of one-day-old hatchling and 40th-stage embryo are increased considerably when maintained in low calcium containing media, whereas the parathyroid gland maintained in high calcium containing media shows a decrease in the cells per microscopic area and the N/C Ratio. However, no remarkable changes were observed in the parathyroid gland obtained from the adult animal (Table 1).

Table 1

The effect of calcium ion on the parathyroid gland of the lizard *Calotes versicolor* maintained in tissue culture

Source of parathyroid	Calcium content of culture media (mm/L)	Mitosis/500 cells	N/C Ratio	Cells/microscopic area
Adult (3)	DW		1/1.26	180± 8.00
Adult (4)	0.50		1/1.22	185±12.00
Adult (4)	1.00		1/1.14	168±13.00
One-day-old hatchling (5)	DW	2.4±0.10	1/1.35	204± 5.00
One day-old hatchling (4)	0.50	0.6±0.08	1/1.16	168±10.00
One day-old hatchling (5)	1.00		1/1.12	164±7.00
40 th-stage embryo (2)	DW	3.4±0.24	1/1.29	214±8.00
40 th-stage embryo (4)	0.50	1.7±0.30	1/1.20	198±5.00
40 th-stage embryo (6)	1.00	1.0±0.09	1/1.23	167±17.00

The values are ± SE

Figures in parenthesis indicate the number of parathyroid glands used.

DISCUSSION

The results of the present investigation clearly show that the secretion of the parathyroid gland is influenced by the surrounding calcium ion concentrations. The parathyroid gland of the embryonic tissue maintained in a low-calcium content shows hyperactivity, whereas a calcium-rich environment causes hypoactivity. These observations are in good agreement with earlier observations made on chick embryo [7].

Although no repert is available regarding the direct measurement of the parathyroid hormone in the lizard, it may be concluded from the present study that release of the parathyroid hormone is stimulated by the low level of calcium ions, whereas a high level of calcium ions inhibits the secretion of the parathyroid hormone. In the previous study, too, I have observed that a low blood calcium content in the lizard *Uromastix hardwickii* increases the activity of the parathyroid gland, whereas high blood calcium levels decrease the activity of the gland [4].

It appears that control of the parathyroid secretion in reptiles is more or less similar to that in mammals.

CONCLUSIONS

Control of the parathyroid gland secretion in the lizard *Calotes versicolor* is studied by using the culture method. A low calcium ion concentration increases the secretory activity of the parathyroid gland, whereas a high calcium ion concentration inhibits the parathyroid secretion.

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NICOTINAMIDE ADENINE DINUCLEOTIDE PYROPHOSPHORYLASE IN FROG LIVER NUCLEI. CYTOCHEMICAL LOCALIZATION, PURIFICATION AND CHARACTERIZATION

BY

R. MEŞTER, D. SCRIPCARIU, A. NEŞTEANU and A. FIXMAN

The presence of NAD pyrophosphorylase in isolated nuclei of frog liver was followed cytochemically using the lead phosphate method. The enzyme was located in association with condensed chromatin, at the nucleolar level and in the nuclear envelope. The enzyme was purified from isolated nuclei by selective solubilization of nuclear proteins with increasing concentrations of salt (0.6 M, 1.2 M and 1.5 M NaCl). The enzymic activity in the 1.2 M NaCl proteic extract of nuclei was 260 times higher than the enzyme activity of the crude nuclear fraction. Some properties of NAD pyrophosphorylase from frog nuclei were evaluated : pH optimum between 7.2 and 8.2, optimum concentration in Mg^{2+} (25 mM), Km for ATP ($9 \times \times 10^{-4}$ M) and Km for NMN ($8,2 \times 10^{-4}$M). The importance of NAD pyrophosphorylase with respect to the synthesis of NAD in frog liver is discussed.

Nicotinamide adenine dinucleotide (NAD) pyrophosphorylase (ATP : NMN adenylyltransferase, EC 2.7.7.1) catalyses NAD synthesis from nicotinamide mononucleotide (NMN) and adenosine triphosphate (ATP). Previous biochemical data on mammalian tissues have shown that the enzyme is localized in the nuclear chromatin and in nucleoli [4], [12], [14], [22]. Although in the literature some data are available concerning the presence of the enzyme in mammalian liver [6], [10], [13], [20], thyroid gland [8], yeast [10], mammary gland [11], fish muscle [15], pigeon erythrocytes [18] and during embryonic development [21], very few works have been produced on the purification of NAD pyrophosphorylase [1], [15], [16].

Despite the importance of pyridine nucleotide in cellular metabolism, little is known about the NAD synthesis in lower vertebrates [15], [17]. Previous data obtained in our laboratory have shown the presence of the enzyme in the frog cocytes and during the first stages of embryonic development [21]. Moreover, we have demonstrated that the administration of nicotinamide enhanced the level of NAD in frog liver [17]. These results have suggested the importance of nicotinamide in the synthesis of NAD via NAD pyrophosphorylase.

In the present work we have followed the activity of NAD pyrophosphorylase from isolated frog liver nuclei, which has not been elucidated yet.

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MATERIALS AND METHODS

All experiments were carried out with pond frogs (*Rana ridibunda* L.) obtained from the lakes around Bucharest.

Isolation of frog liver nuclei. Nuclei from frog liver were isolated by the procedures described by Chauveau et al. [3] and Busch and Daskal [2], with some modifications. The frog liver (30 g) was minced and washed with saline solution of 0.65% NaCl at cold. The tissue was suspended in 10 volumes of 0.25 M sucrose containing 3.3 mM $CaCl_2$ and was homogenized in a glass homogenizer. The homogenate was filtered through two layers of gause and centrifuged at 1000 g for 10 min. The nuclear pellet was resuspended in the same medium and the homogenization was repeated. The sediment of crude nuclei was considered initial nuclear fraction. Observations by phase-contrast microscopy showed that the nuclei were well preserved. Subsequently, the crude nuclear fraction was resuspended in 0.25 M sucrose with 3 mM $CaCl_2$ and layered over a discontinuous gradient of sucrose (1.4–2.0 M) and centrifuged at 35.000 g for 60 min. The purified nuclei were resuspended in potassium phosphate buffer 1 mM, pH 7.4 up to the concentration of 0.5 M sucrose, and afterwards were pelleted by centrifugation at 6000 g for 10 min. Finally, the nuclei were suspended in 1 Mm potassium phosphate buffer, pH 7.4 with 0.25 M sucrose.

Part of the purified nuclei were processed for cytochemical identification of the enzyme and were suspended in Tris-acetic acid buffer 50 mM, pH 7.6 with 0.25 M sucrose.

Solubilization of NAD pyrophosphorylase from isolated frog liver nuclei. Nuclei were first extracted with 0.6 M NaCl prepared in potassium phosphate buffer 1 mM, pH 7.4, containing 0.25 M sucrose, for 20 min, by swirling with a magnetic stirrer at cold (4°C). The nuclear suspension was centrifuged at 10.000 g for 15 min, and the supernatant obtained was considered as 0.6 M NaCl nuclear proteic extract. The sediment was subjected to the next extraction with 1.2 M NaCl, in the same conditions. Finally, the nuclear precipitate was extracted with 1.5 M NaCl. The activity of NAD pyrophosphorylase in the nuclear proteic fractions, extracted with solutions of different ionic strength, is presented in Table 1.

Table 1

Selective solubilization of NAD pyrophosphorylase in isolated nuclei of frog liver with solutions of different ionic strength

Steps	Protein mg/ml	Enzymic activity nmol/mg protein/min
Crude nuclear fraction	2.5	0.12
0.6 M NaCl proteic extract	0.46	9.70
1.2 M NaCl proteic extract	0.10	31.65
1.5 M NaCl proteic extract	0.11	14.00

Electron microscopic study of NAD pyrophosphorylase. Purified nuclei were prefixed in 2% formaldehyde, buffered with Tris-acetic acid 50 mM, pH 7.6 containing 0.25 M sucrose and 1 mM magnesium acetate, for 15 min. The nuclei were washed several times with the same buffer without formaldehyde, and incubated in the following incubation medium: NMN 2 mM, ATP 2 mM, nicotinamide 0.2 M, magnesium acetate 10 mM, lead acetate 1 mM, sucrose 0.25 M in Tris-acetic acid 50 mM, pH 7.6. After incubation (60 min at 37°C), nuclei were washed with Tris-acetate buffer, fixed in 1.5% glutaraldehyde buffered with cacodylate 50 mM, pH 7.6, for 30 min. Nuclei were postfixed in 1% OsO_4 solution buffered with cacodylate, for 1 hour. Nuclei were washed with cacodylate buffer and fixed in 0.5% agar. The small pieces were dehydrated in the series of alcohol and embedded in Epon. For control, the nuclei were processed as above, but incubated without substrate. The sections were cut on a LKB ultramicrotom and examined with a Philips 201 electron microscope.

Determination of NAD pyrophosphorylase activity. The NAD pyrophosphorylase activity was determined by the method described by Greenbaum et al. [10]. The standard incubation medium contained: glycylglycine buffer 50 mM, pH 7.6, NMN 2 mM, ATP 6 mM, $MgCl_2$, 15 mM, nicotinamide 0.2 M and varying concentrations of proteic extract. In parallel, control samples were processed as above, but with the omission of NMN. The values of control assays were subtracted from experimental values. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped with 1 N perchloric acid. The mixture was neutralized with KOH and the supernatant obtained after centrifugation at 10.000 g for 10 min was utilized for NAD determination.

The amount of NAD formed was measured according to the method of Slater et al. [23]. The activity of the enzyme was expressed in nmoles of NAD formed per mg protein per min.

The protein content was estimated spectrophotometrically at 260 and 280 nm, after the method of Warburg and Christian [24].

RESULTS

Cytochemical localization of NAD pyrophosphorylase in isolated frog liver nuclei. Several authors have employed lead as a capture agent for cytochemical localization of adenylate cyclase, in which pyrophosphate was precipitated in the presence of a lead salt [5], [7]. A similar procedure for the identification of NAD pyrophosphorylase was employed. To obtain information about the inhibition induced by lead on the enzyme activity, we followed the enzymic activity in the presence of varying concentrations of lead acetate. At the concentration of 1 mM lead acetate in the assay probe, about 30% of the enzyme activity was inhibited, nevertheless it may afford cytochemical identification of the enzyme.

As illustrated in Fig. 1, the cytochmical reaction product (lead pyrophosphate) is widely distributed in association with condensed chromatin of frog liver nuclei. The density of the reaction product appears to be much higher at the level of the nucleoli (Fig. 1 arrow). A high number of precipitates were found in the nuclear membranes (Fig. 1, arrows). The inner nuclear membrane showed some positive reaction, indicating the heterogenous distribution of the enzyme in the nuclei. Biochemical studies on mammalian liver have shown that NAD pyrophosphorylase is exclusively located in chromatin [12], [14], [22]. This conclusion is sup-

ported by the present electron microscope observation. The identification of the enzyme in nuclear envelope has not been reported before.

Purification of NAD pyrophosphorylase from frog liver nuclei. Fractionation of nuclear proteins by extraction with solutions of different ionic strength is largely used in the study of chromosomal proteins [9]. Taking into account that NAD pyrophosphorylase is attached to nuclear chromatin, we followed the solubilization of the enzyme by selective extraction of proteins with three different concentrations of NaCl (Table 1). It should be pointed out that the enzyme was extracted from the nuclei at all three concentrations of NaCl employed. The highest specific activity of NAD pyrophosphorylase was obtained in 1.2 M NaCl proteic extract, with an estimated value of 31,6 nmoles NAD formed per mg protein per min. The enzyme from the proteic extract solubilized with 1.2 M NaCl was 260 times more active than the enzyme from the crude nuclear fraction.

The solubilization of NAD pyrophosphorylase from the nuclei in the presence of detergent (Triton X–100 in concentration of 1%) did not give a more efficient extraction of the enzyme. The enzyme solubilized from the frog nuclei appears, to be instable. A significant loss of activity was detected during storage at 2°C, for 1–2 days.

Some properties of NAD pyrophosphorylase from frog liver nuclei. Effect of incubation time. The effect of the incubation time on the synthesis of NAD was followed with a crude nuclear fraction and nuclear proteic extract. In both cases, the activity of the enzyme was linear through a reaction time of 60 min, under standard experimental conditions.

Effect of metal ions. NAD pyrophosphorylase from frog liver nuclei requires metal ions for its full activation. More than 40% of the activity was found in the absence of Mg^{2+} ions. Addition of $MgCl_2$ to the assay mixture brings about an over 60% increase of activity. Full activation of the enzyme was obtained in the presence of 25 mM $MgCl_2$. The enzyme from frog nuclei requires higher concentrations of divalent cations in comparison with NAD pyrophosphorylase from other tissues [10], [15], [18].

pH optimum. NAD pyrophosphorylase from frog liver nuclei has a broad pH optimum, ranging between 7.2 and 8.2. Therefore all subsequent experiments were carried out at pH 7.6. These values are in agreement with early observations on the enzyme obtained on other biological sources [1], [15], [16], [20].

Kinetic studies. The Michaelis-Menten constants for NAD pyrophosphorylase towards NMN and ATP substrates were determined. Double reciprocal plots of these data gave Km values for NMN and ATP of $8,2 \times 10^{-4}$ M, and 8×10^{-4} M, respectively (Figs [2] and 3). Higher concentration of the substrates did not affect the maximal velocity of the enzyme. The Michaelis- Menten constants of the enzyme from frog liver nuclei are higher than those previously reported on skipjack muscle [12], hog liver [16] and rat liver [10]. The discrepancy is probably due, in part, to peculiarities of NAD pyrophosphorylase from different tissues, reflecting the affinities of substrates towards the active sites of the enzyme.

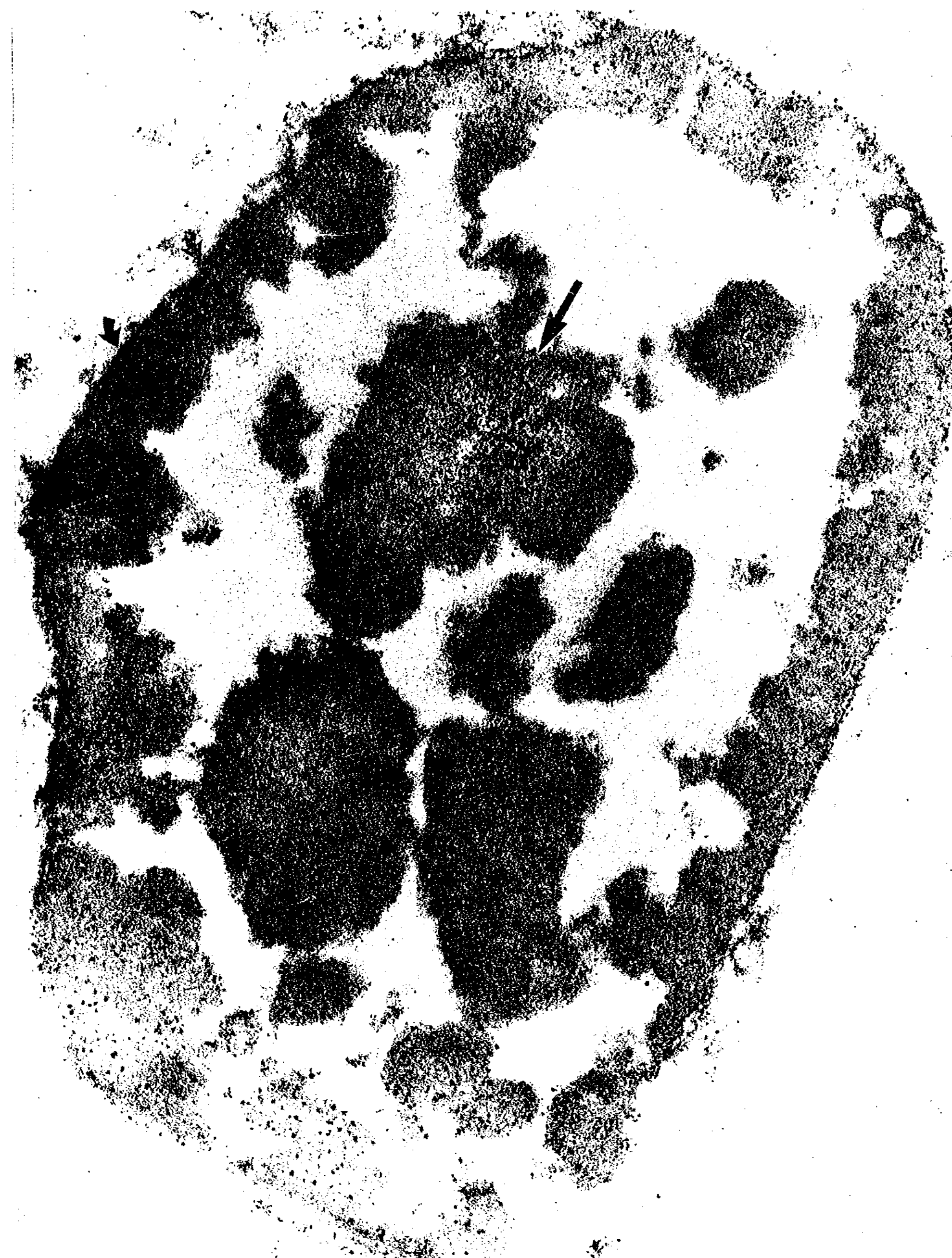

Fig. 1. — Cytochemical localization of NAD pyrophosphorylase activity in isolated nuclei of frog liver. The enzymatic reaction product was revealed in condensed chromatin, in nuclear membranes (arrows) and at the level of nucleoli (arrow). Uncolored preparation. Magnification 1700 ×.

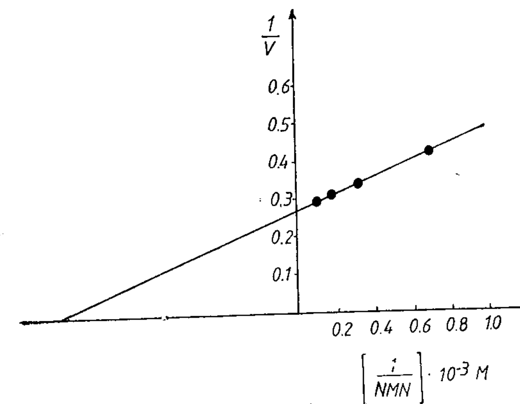

Fig. 2. — Lineweaver-Burk plot of NAD pyrophosphorylase activity as a function of NMN concentration.

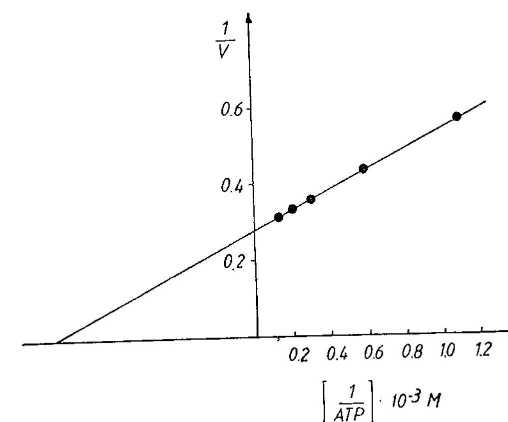

Fig. 3. — Lineweaver-Burk plot of NAD pyrophosphorylase activity as a function of ATP concentration.

DISCUSSION

Very few studies concerning the purification of NAD pyrophosphorylase have been reported. The first purification procedure of the enzyme was described by Kornberg [16], who obtained a partial purified enzyme from yest and hog liver. Recently, NAD pyrophosphorylase from the nuclear fraction of fish muscle has been purified and characterized [15]. The authors obtained a purified preparation which had a specific activity of about 35 nmoles NAD formed per mg protein and per min, a value which is very close to the specific activity of our preparation.

The presence of the enzyme in all the nuclear proteic fractions is the result of the degree and type of functional integration of this protein in the nuclei. Solubilization of the enzyme with solution of different ionic strength, supports the suggestion that the nuclear chromatin represents a dynamic structure, in which interaction of nuclear nonhistone proteins with DNA and histones forms temporary bonds of variable strength.

Several data have been reported in the literature concerning the activity of NAD pyrophosphorylase in the total proteic extract from different types of tissues [4], [10], [11], 13], [19], [20], but the enzymatic activities were expressed in different ways. To facilitate some comparisons, data taken from the works of Greenbaum et al. [10] and Kono et al. [15] have been summarized in Table 2 together with our results concerning the

Table 2

The activity of NAD pyrophosphorylase in the total proteic extract of several tissues

Tissue	Enzymatic activity
Crude nuclear fraction of fish skeletal muscle	0.058 nmol/mg/min *
Crude nuclear fraction of frog liver	0.12 nmol/mg/min
Crude homogenate from rat liver	2.10 nmol/min/0.1 ml **

* = recalculated from the data of Kono et al. [15].

** = recalculated from the data of Greenbaum et al. [10].

activity of the enzyme in crude nuclear fraction. The activity of NAD pyrophosphorylase in the crude nuclear fraction of frog liver is 0.12 nmoles/mg protein/min, value which is about 20 times lower than the enzyme activity in rat liver homogenate [10], but two times higher than the enzyme activity in the crude nuclear fraction of fish muscle [15]. One interpretation may be that, in frog liver nuclei, the rate of NAD synthesis is decreased. The decreased rate of pyridine nucleotide synthesis is in accordance with the low level of NAD in frog liver. The content of total NAD (NAD^+ + NADH) in frog liver is 10—20 times lower than in the liver of mammals [17].

The concentration of NAD in the cell is a dynamic phenomenon which is dependent on many factors: the activity of the enzymes involved in its synthesis and degradation, the concentration of the enzyme, the availability of substrates, cofactor and other metabolites which may modulate the velocity of pyridine nucleotide synthesis. Our observations on the activity of NAD pyrophosphorylase in frog liver nuclei, must be considered in the light of current knowledge concerning the role of substrates. It was suggested that the activity of the enzyme in rat liver is several times higher than needed [6], [10]. The administration of nicotinamide increases the amount of NAD in frog liver 3 times and 10–20-times in the rat liver [13], [17]. This enhancement may by regarded as dependent on the availability of NMN. The second factor which might interfere with the rate of NAD synthesis is the availability of ATP. However, high concentrations of ATP did not affect the rate of NAD synthesis, either in mammalian liver [10] or in frog liver (our data). It is important to note that the activity of NAD pyrophosphorylase in the nuclear chromatin may be dependent on the nuclear proteins organization. Therefore, NAD synthesis appears to be under complex control, dependent on cell cycle phases and other metabolic factors which interfere with chromatin organization.

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DISACILIN INFLUENCE UPON BLOOD SERUM LIPIDS AND CHOLESTEROL

BY

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Disacilin determines an increase of total serum lipids in rabbits during their growth period and a decrease during the adult stage. Total cholesterol and its fractions concentrations in the blood are reduced, and the amplitude of the effect depends on the mode of administration, too. In the case of i.m. administration and for the same dose, the effects are more evident than in the case of the oral one. There have been ascertained positive effects as regards the biostimulative action of the analysed product.

Besides their well-known energetic role, lipids have important plastic roles as well, either by intervening in protein synthesis [20], or by directly participating in forming membrane structures.

The relation between the anabolism and catabolism of lipids in ontogenesis varies, at different stages, from species to species [23]. In mammals, a quantitative increase of lipids occurs especially in the more advanced stages of embryonal evolution [11], [13], [17], [21], [24], [25]. Central nervous system lipids increase very much during the post-natal period in connection with the myelogenic process [4], [9], [15]. Hepatic lipids appear very early and accumulate in high amounts up to the birth moment [10], and decrease after that, being ensured by lactation. A high accumulation of lipids in the regions with intense morphogenesis [6] as well as an evident increase of lipemia in females during gestation [19], have been noticed.

In the post-natal period, total lipids in the body as well as those in the blood increase continually, first more rapidly and then at a slower rate. An important delay of growth in the case of lipid-free diets has been observed, since certain fat acids cannot by synthesized by the body [7].

The adult stage characterstic values of total lipids and their various fractions are recorded at the same time with somatic maturation, the liver being responsible for the general equilibrium of lipids [8].

With the advancement in age, a slow increase of serum lipids can be recorded. Their concentration, in old age, can reach a value even twice as high as that in the adult, but not exceeding usually 50 % of the latter [18].

Cholesterol, in its turn, plays an important part not only in the life of the adult organism but also in the processes of growth and development. In mammals, during the post-natal period of growth, total serum cholesterol increases continually both by its free fraction and by the esterified

one. In an initial phase values increase rapidly, almost tripling themselves, subsequently slowing down to the characteristically adult level [19].

With the progress in age, total serum cholesterol increases slowly [1], reaching maximum values in the old age [18]. The modification of the exchange of cholesterol between cells and plasma depends on the modification of the ratio between the esterified cholesterol fraction and the free one in the blood [14]. The increase of blood cholesterol by way of exogenous contribution leads to the depression of its hepatic synthesis [3]; the reduction of the exogenous contribution leads to the stimulation of cholesterol synthesis [5].

The complex participation of lipids in the process of growth justifies the study of disacilin effects as it is a product with biostimulative characteristics.

MATERIAL AND METHODS

The experiments were performed upon four-month-old Chinchilla rabbits having a body weight around 2 kgs each at the beginning. The whole observation period (12 months) was extended over the second stage of growth of the animals (from 4 to 10 months of age) and over the mature stage (from 10 to 16 months of age).

The treatment with disacilin was permanent, being given to a group of twenty animals by ingestion (6.25 mg/kg b.wt./day) and to another group of twenty in i. m. injections (5 mg/kg b. wt./day). A control group of twenty animals did not receive disacilin at all.

Analyses were made before the beginning of the treatment, two weeks after that, and then at one-month intervals in the whole period of treatment. The measuring of total lipids was made by the Woodman-Price method [26] and of cholesterol and its fractions by the Georgescu-Constantinescu method [12].

RESULTS

A. Serum lipids. In the animals not treated with disacilin, the total lipids concentration shows a slight increase in time, the average value in the first interval (251.02 mg %) being by 4.15 % higher than the initial value (241.0 mg %), and that of the second half of the interval (265.53 mg %) by 5.76 % higher than the one in the previous period (Fig. 1, I–TL).

In the animals treated with disacilin per os, the total lipids present ample oscillations in the first two months, with a clear tendency to increase in the first month and a clear subsequent evolution towards values smaller than the initial one (Fig. 1, II–TL). In the second part of the interval, the reduction of lipids concentration continues with no unusual oscillations, the average value corresponding to it (232.0 mg %) being thus smaller than that corresponding to the first part of the interval (253.40 mg %) and that recorded in control animals in this stage (265.53 mg %).

In the animals treated with disacilin in i. m. doses an evident increase of total serum lipids takes place in the first six months. The mean of the values (319.48 mg %) is thus higher than in the group treated per os as

well as in the control one. This increase still persists at the beginning of the second half of the interval and is then rapidly replaced with an evident reduction (average value 222.10 mg %) below the value in the group treated per os (232.32 mg %) and much under the value in the control group (265.53 mg %) (Fig. 1, III–TL).

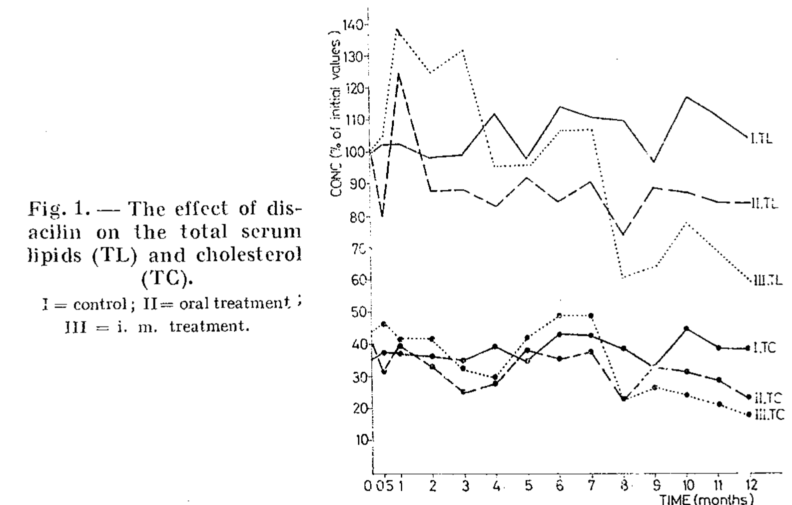

Fig. 1. — The effect of disacilin on the total serum lipids (TL) and cholesterol (TC).
I = control; II = oral treatment; III = i. m. treatment.

B. *Serum cholesterol.* In the animals not treated with disacilin, the total serum cholesterol presents a slow increase along the whole period of observation (Fig. 1, I–TC). In the first six months a rise of its level by 2. 95 % as compared to the initial value is recorded, and in the following

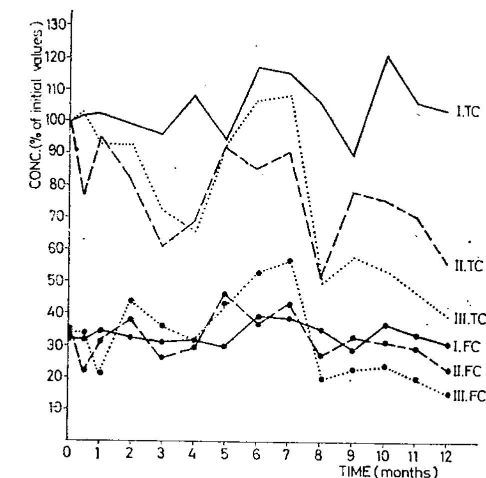

Fig. 2. — The effect of disacilin on the free serum cholesterol (FC).
I = control; II = oral treatment; III = i.m. treatment.

six months there is a continuation of this growth by 5.67 % compared to the previous period. These increases are achieved both at the expenss of the free fraction of cholesterol (Fig. 2, I–FC), by 1.69 % in the first stage

and by 5.05 % in the second one, and of the esterified fraction (Fig. 3, I—EC), by 3.53 % in the first stage and by 5.95 % in the second one. During the whole period of observation the EC/FC ratio is around 2.

In the case of animals treated with disacilin per os, in the first six months, total cholesterol presents a rather evident tendency of reduction (ca 20 % on the average as compared to the initial value), which is to continue in the following six months as well (by 12 % on the average as compared to the previous stage) (Fig. 1, II—TC). The free fraction follows generally the total cholesterol variation curve (Fig. 2, II—FC), like the esterified one, but which presents in general more important modifications (Fig. 3, II—EC). In the first month, the EC/FC ratio maintains itself at the normal value (2.08), but subsequently decreases and stops at a much smaller and relatively constant value for the rest of the observation period (average value 1.22).

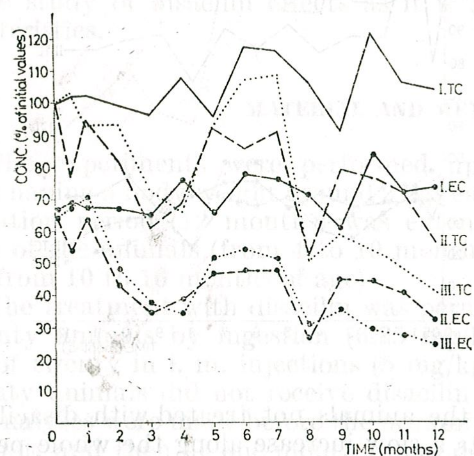

Fig. 3. — The effect of disacilin on the esterified serum cholesterol (EC).
I = control; II = oral treatment; III = i.m. treatment; TC = total cholesterol.

In the animals treated with disacilin in i.m. injections, similar but more evident effects could be observed. In the first six months the ample increasing variation at the end of the period, makes the average value of total cholesterol in this interval decrease by only 9.32 % below the initial value. In the second part of the interval the decreasing variations are more evident and this makes the mean of the values to be by 27 % under the one corresponding to the previous stage (Fig. 1, III—TC). The free fraction increases in the first period by 6.30 % above the initial value and decreases very evidently in the second period, by 18.55 % as compared to the anterior stage (Fig. 2, III—FC). On the other hand, esterified cholesterol decreases in the first six months by 16.75 % on the average and even more in the following six months, by 32.94 % on the average (Fig. 3, III—EC). The EC/FC ratio maintains itself in the first month close to the normal value (2.37). Subsequently it goes down to a value slightly over unity (1.21), insignificantly smaller than the one noticed in the case of the treatment with disacilin per os (1.22).

DISCUSSIONS AND CONCLUSIONS

The results of the experiments prove that disacilin exercises a positive influence with a view to biostimulation in the way it influences serum lipids and no doubt the whole lipid metabolism.

The rise of serum lipids ensures a higher level of the development process than in the animals that were not treated, in the first period of growth. By this effect disacilin ensures a rich energetic and structural material just at the moment when the organism mostly needs it.

In the second period of growth, the effect of disacilin of reducing lipids is also positive and it expresses the creation of the possibility to delay (the onset of old age phenomena and the continuation of growth. It can also be admitted a more intense use of lipids than in the animals not treated, as a direct energetic material and probably their better use in glycogenesis.

In general, for the same dose, the effects of disacilin upon serum lipids are more comprehensive and prolonged after i. m. administration than per os.

The same thing can be observed with total cholesterol, with the difference that in this case no matter how disacilin is administered, it determines an evident cholesterol reduction in the blood. The esterified and the free fraction of serum cholesterol present a decrease in concentration and a reduction of their ratio to slightly over unity.

Since cholesterol is a precursor of bile acids synthesis [22] which in their turn are an important factor of intestinal lipid absorption, we could admit that the effects of disacilin treatment upon the blood cholesterol level (expressing, among other things, its intensive use in the increased hepatic synthesis of bile acid) may lead to a better absorption of lipids during intense body development in animals.

On the other hand, the modification of the ratio of esterified free cholesterol fractions and its maintenance at a low value which is not below unity, accounts for lower serum lipid concentrations than in non-treated animals, and their maintenance in time.

Disacilin influence upon the blood cholesterol and its fractions represents a positive effect in regard to the biostimulative qualities of the analysed product.

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L'INFLUENCE DU TRAITEMENT AU POLIDIN SUR LE REPEUPLEMENT DE LA RATE ET LA SÉCRÉTION DU MIF CHEZ LES SOURIS IRRADIÉES

PAR

MARIA-SENA CRIVII, Z. URAY et I. KIRICUŢĂ

Using the migration inhibition test *in vitro* in irradiated and Polidin treated mice we have shown the migration capacity of cells in the splenic explant and the lack of migration inhibition factor (MIF).
The animals treated with Polidin 6 days after irradiation exhibit the repopulation of the spleen, as shown by the increase of migration capacity of the splenic cells. We did not find the production of the migration inhibition factor by these cells. The behaviour of irradiated and Polidin treated animals was similar to that of control nonirradiated and treated animals.

Dans les dernières 15 années les produits immunostimulateurs ont été le sujet des nombreuses études d'intérêt expérimental et clinique et les méthodes de l'immunothérapie non spécifique sont à cette heure les plus employées. Ces méthodes mettent en jeu soit des agents microbiens [3], [9], [10] soit des extraits thymiques [4], [14], [15] soit des produits synthétiques, par exemple, le Lévamisole [1], [12].

Les recherches effectuées à l'Institut Oncologique de Cluj-Napoca [7], [8] ont mis en évidence la capacité du Polidin (préparation polymicrobienne) de réduire et de retarder d'une manière significative le processus de constitution des métastases de quelques tumeurs expérimentales chez les rongeurs, phénomène attribué à ses propriétés immunostimulatrices non spécifiques.

Le Polidin, comme le Lévamisole, possède la capacité d'atténuer les modifications morphologiques destructrices de la rate et du thymus, qui sont directement impliqués dans les mécanismes immunitaires de l'organisme [6], [14].

Basés sur ces données, nous avons utilisé le test de l'inhibition directe de la migration des cellules spléniques pour obtenir des indications cytophysiologiques sur les éléments cellulaires impliqués dans ce procès à la suite d'un traitement des souris irradiées et traitées au Polidin.

MATÉRIEL ET MÉTHODE

Les expériences ont été effectuées sur des souris DBA, mâles, d'un poids de 20 $\pm$ 1 g, soumises à un régime standard.

Les animaux ont été partagés en 4 lots :
(a) témoins non traités
(b) témoins traités
(c) irradiés non traités
(d) irradiés traités

L'irradiation a été effectuée en boîtes spéciales en plastique sous un appareil de radiothérapie TUR—I (180 kV, 10 mA, filtre 1 Cu, DEF = 40 cm, débit 23 R/min), la dose totale étant de 400 rad.

Après l'irradiation les animaux ont été injectés i.p. avec 0,5 ml/kg Polidin * [11] pendant 6 jours. Trois jours après, les animaux ont été sacrifiés.

Le test de l'inhibition directe de la migration a été effectué d'après la méthode de Svejcar [13]. On a employé le milieu de culture IC—65 enrichi en sérum homologue inactivé à 56°C, 30 minutes, des antibiotiques et du Polidin en concentration de 50 µl/ml.

Après la projection de surface de migration sur papier Wattman 1, avec un microscope MC—1, les résultats exprimés en mg ont été calculés pour leur signification statistique par le test «t» de Student.

L'index de la migration a été calculé d'après la formule suivante :

$$IM = 100 \times \frac{\text{surface de migration en présence de Polidin}}{\text{surface de migration en l'absence de Polidin}}$$

La positivité du MIF commence pour $p < 0{,}05$. On a introduit également le coefficient de corrélation qui peut nous montrer si les deux phénomènes observés (la migration et l'inhibition de la migration) sont interdépendants.

RÉSULTATS ET DISCUSSIONS

Les résultats obtenus sont présentés dans le Tableau 1 et les Figures 1 et 2.

Le Tableau 1 montre que les témoins non traités ont une moyenne des surfaces de migration de $\overline{X} = 152{,}66$ et l'index de migration IM = 100,56 %, n'indique aucune influence significative ($p > 0{,}25$) si le Polidin est ajouté au milieu de culture.

Les témoins traités pendant six jours ont une moyenne de 137,25 mg et IM = 105,06, ce qui indique que le facteur de l'inhibition de la migration (MIF) n'a pas été sécrété, c'est-à-dire qu'il ne s'agit pas d'une réponse lymphocytaire splénique ($p > 0{,}5$).

Les souris irradiées et non traitées présentaient des surfaces de migration diminuées $\overline{X} = 76{,}33$ mg, ce qui montre une déplétion en cellules migratoires et/ou le baissement de la capacité physique de migration. De plus, chez ces animaux, l'inhibition de la migration, c'est-à-dire la sécrétion du facteur lymphocytaire (MIF) n'est pas présente ($\overline{X} = 77{,}33$; IM = 101,79 %, $p > 0{,}5$).

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* Polidin : suspension polymicrobienne billiée dans un concentration de 48 = 10[4] organismes/ml, produit de l'Institut « Dr. I. Cantacuzino » Bucarest, ayant un effet non spécifique de stimulation du système réticulo-endothélial.

Tableau 1

La moyenne de la migration, l'index de migration (IM %), le coefficient de corrélation et la pente de régression chez les souris irradiées et traitées au Polidin

GROUPE	La moyenne de la migration		L'index de migration (IM %)	Le coefficient de corrélation „r" et la pente de régression
	Sans P	Avec P		
1. Témoins non-traités (n = 5)	162,75	174,25	107,00	r = 0,416
	173,00	163,25	94,36	y= 0,25x+75,71
	146,00	153,25	104,90	t = 0,792
	146,50	151,00	103,00	p <0,5
	160,25	150.50	93,90	
	$\overline{X}$=157,70	158,45	100,56	
	ES= 5,75	5,11	3,11	
	t= 1,44	p<0,25		
2. Témoins traités au Polidin (n = 5)	125,75	143,75	114,30	r = 0,662
	136,25	135,00	99,08	y = 0,34x + 96,01
	158,50	150,50	94,95	t = 1,529
	141,75	150,75	106,30	p <0,25
	124,00	137,25	110,70	
	$\overline{X}$=137,25	143,45	105,06	
	ES= 6,98	3,65	4,00	
	t = 0,76	p <0,50		
3. Irradiés non-traités (n=6)	86	81	94,18	r = 0,49
	74	74	1000,00	y = 0,23 x + 59,65
	69	80	115,94	t = 1,12
	78	76	97,43	p <0,5
	81	79	97,53	
	70	74	105,71	
	$\overline{X}$=76,33	77,33	101,79	
	ES= 2,70	1,26	3,24	
	= 0,93	p> 0,25		
4. Irradiés traités au Polidin (n = 6)	174,33	112,00	64,24	r = 0,224
	129,66	131,66	101,54	y = 0,14x + 115,74
	150,33	149,33	99,33	t = 0,459
	159,00	145,00	91,19	p <0,5
	184,33	152,66	82,81	
	195,66	150,33	76,83	
	$\overline{X}$=165,55	140,16	85,99	
	ES= 9,86	6,43	7,11	
	t = 2,16	p> 0,05		

* Chaque valeur représente la moyenne pour 3 ou 4 surfaces de migration ; $\overline{X}$ = la moyenne ES= l'erreur standard.

Les cellules spléniques des animaux irradiés et traités au Polidin présentent une capacité de migration augmentée ($\overline{X} = 165{,}55$) semblable aux

témoins non traités. Mais, la sécrétion du MIF est vaguement exprimée $\overline{X}$ = 140,16 mg (IM = 85,99 %, p = 0,05).

Ce phénomène peut être mieux étudié si on calcule les coefficients de corrélation entre les surfaces de migration en absence et en présence du Polidin, ainsi que la pente de régression linéaire. De cette manière nous avons obtenu des coefficients de corrélation au-dessus de 1 (0,224—0,662) ce qui demontre qu'il n'y a pas des corrélations entre les deux phénomènes.

Basés sur les données de la littérature, nous pouvons supposer que l'augmentation de la surface de migration peut être provoquée par l'augmentation du nombre de cellules migratoires d'une part, et par l'augmentation de leur capacité physique de migration d'autre part. Ainsi que, Uray et coll. [14], Uray [15] ont constaté une déplétion lymphocytaire accentuée dans la rate des souris irradiées, ayant comme conséquence la diminution et même la disparition des follicules lymphoïdes et l'estompement de la structure caractéristique de l'organe. Ces animaux présentent une petite capacité de migration (49 % de la valeur du témoin). Le traitement au Polidin atténue les modifications morphologiques destructrices de la rate [6] et augmente le nombre des cellules polynucléaires dans le sang périphérique [5]. Ces modifications morphologiques de la rate viennent d'expliquer l'augmentation des aires de migrations chez les souris irradiées et traitées au Polidin.

Si on fait la comparaison entre l'effet du Polidin et l'effet de la Leucotrophine sur le repeuplement de la rate des souris irradiées avec des cellules fonctionnelles, on constate que les deux immunostimulateurs apportent le redressement morphologique de la rate, mais seulement la léucotrophine stimule la sécrétion des facteurs lymphocytaires [2].

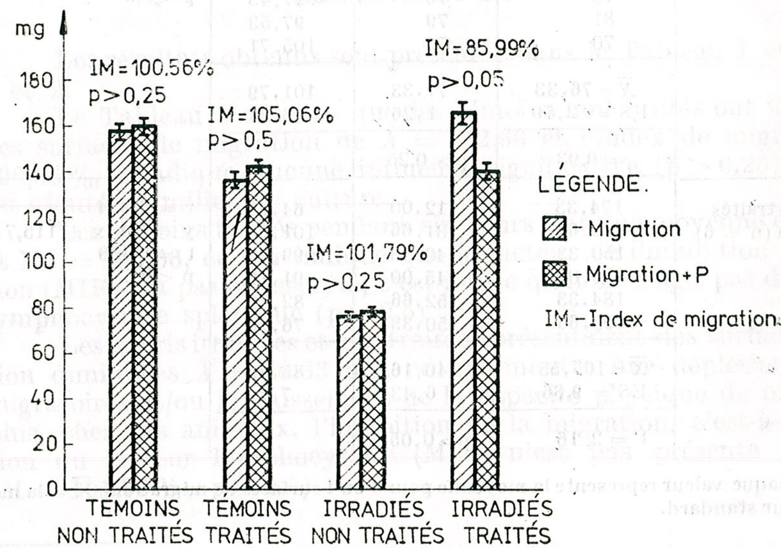
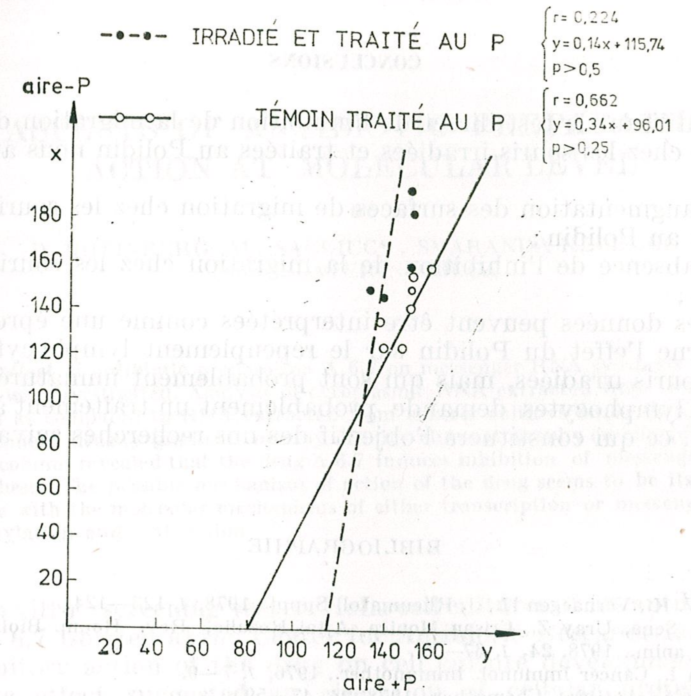


Fig. 1



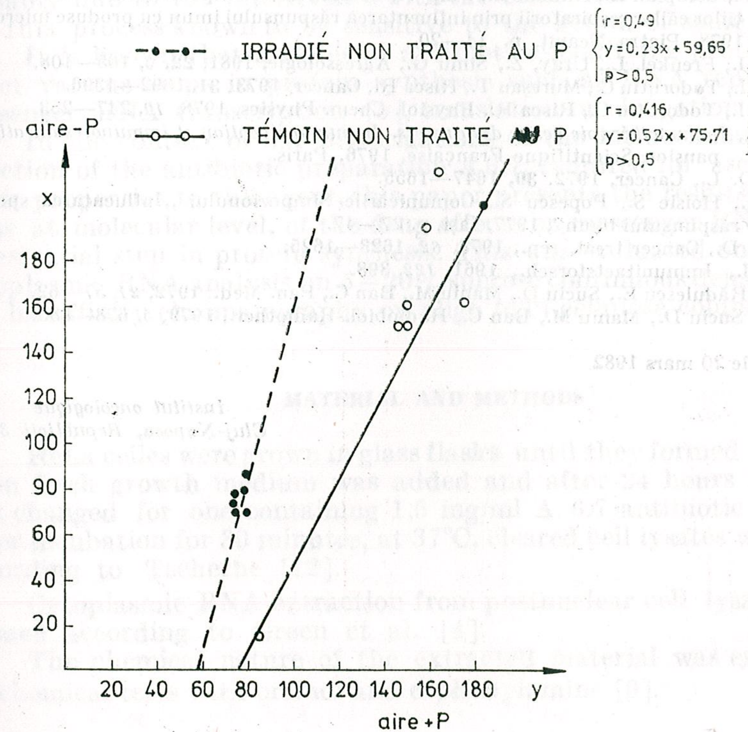


Fig. 2

CONCLUSIONS

En utilisant le test direct de l'inhibition de la migration des cellules spléniques chez les souris irradiées et traitées au Polidin nous avons constaté :

— l'augmentation des surfaces de migration chez les souris irradiées et traitées au Polidin ;

— l'absence de l'inhibition de la migration chez les souris irradiées et traitées ;

— ces données peuvent être interprétées comme une épreuve en ce qui concerne l'effet du Polidin sur le repeuplement lymphocytaire de la rate des souris irradiées, mais qui sont probablement immaturées. L'activation des lymphocytes demande probablement un traitement à la longue au Polidin, ce qui constituera l'objectif des nos recherches suivantes.

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SOME ASPECTS OF ANTIBIOTIC PREPARATION A 6.7 ACTION AT MOLECULAR LEVEL

BY

P. ROTINBERG, AL. SAUCIUC*, SMARANDA KELEMEN, and ECATERINA DUCA

The effect of antibiotic preparation A 6.7 on messenger RNA synthesis in HeLa cells was investigated. Analysis of cytoplasmic RNA extracted from treated cultures as compared to RNA extracted from control cultures on 5—20 % sucrose continuous density gradients and by affinity chromatography on oligo(dT) cellulose column revealed that the drug A 6.7 induces inhibition of messenger RNA synthesis. The possible mechanism of action of the drug seems to be its interference with the molecular mechanisms of either transcription or messenger RNA adenylation and maturation.

"In vitro" screening test on HeLa cell cultures of an antibiotic preparation A 6.7 isolated at the Center for Antibiotic Research Iaşi, indicated the inhibitory action of the drug on cell culture development [10]. This cytostatic effect suggested an alteration of cellular protein synthesis probably due to the interference with the molecular mechanisms regulating this process known to be sensitive to the action of some antibiotics.

It is known that a series of antibiotics modifying protein synthesis affect various steps in protein synthesis such as DNA replication [11], messenger RNA transcription [5], translation or elongation [2].

In the context of our investigations of the intracellular mechanism of action of the antibiotic preparation A 6.7, required for ascribing cytostatic properties to this agent, this paper presents the results of investigations at molecular level, of the drug effects on messenger RNA synthesis, an essential step in protein synthesis. This was evaluated on the basis of cytoplasmic RNA analysis on 5—20 % sucrose continuous density gradients and by affinity chromatography on oligo (dT) cellulose columns.

MATERIAL AND METHODS

HeLa celles were grown in glass flasks until they formed a monolayer. Then fresh growth medium was added and after 24 hours this medium was changed for one containing 1.5 mg/ml A 6.7 antibiotic preparation. After incubation for 30 minutes, at 37°C, cleared cell lysates were prepared according to Tscherne [12].

Cytoplasmic RNA extraction from postnuclear cell lysates was performed according to Green et al. [4].

The chemical nature of the extracted material was established by biochemical tests with orcinol and diphenylamine [9].

The quantitative estimation of RNA obtained from control and drug-treated cultures as an expression of the level of its synthesis was performed by recording the UV absorbance at 260 nm of 10 μl RNA solution/ml tri-distilled water in a Beckman spectrophotometer.

RNA analysis on sucrose gradients [8]. 5–20% density continuous sucrose gradients (RN-ase activity free) in 5 ml tubes were prepared. RNA samples, obtained from control and treated HeLa cell cultures, were applied directly on the gradients which were centrifuged 120 min at 36,000 rpm, 2°C in a swingout rotor of VAC–601 ultracentrifuge. The gradients were fractioned and fraction absorbance at 260 nm was measured in a Beckman spectrophotometer.

RNA analysis by affinity chromatography on oligo (dT) cellulose column [6]. 10 A_{260} units each from different RNA preparations in high salt buffer 0.1 M Tris-HCl (pH 7.5), 0.5 M KCl were applied to a 0.6 ×4 cm oligo (dT) cellulose column, previously equilibrated with the same buffer. After the sample had entered the column, the latter was washed with high salt buffer until the absorbance at 260 nm of the effluent was zero and oligo (dT) cellulose bound material was eluted with 0.01 M Tris-HCl (pH 7.5) without KCl.

The absorption at 260 nm of the 1 ml column fractions was determined with the aid of a Beckman spectrophotometer.

RESULTS AND DISCUSSIONS

Biochemical tests indicated the extracted material from HeLa control and drug treated cultures to be orcinol positive and diphenylamine negative, thus representing RNA and not possible contaminating DNA which might be present in the cleared lysates.

The absorbance registered at 260 nm was 0.065 for RNA extracted from control cultures and 0.035 for RNA extracted from drug-treated cultures. This low value shows a significantly lower (about 50 per cent) amount of RNA, suggesting an inhibition of its synthesis induced by the antibiotic preparation A 6.7 in HeLa cells.

RNA analysis on 5–20% sucrose continuous density gradients revealed characteristic sedimentation profiles which are shown in Fig. 1 and Fig. 2.

The sedimentation profile of RNA extracted from control cultures as illustrated in Fig. 1, is characterized by an alternation of absorbance peaks corresponding to RNA of different sizes, that is from material of low molecular weight located in the upper part of the gradient, to RNA of higher size, in the lower part of the gradient. The peak corresponding to the middle part of the gradient, (that is fractions 7–12) represents messenger RNA.

The sedimentation profile of RNA extracted from HeLa cell cultures incubated with the drug A 6.7, illustrated in Fig. 2 shows the persistence of the absorbance peaks corresponding to material of low and high weight, a marked decrement of the messenger RNA peak (fractions 8–10). This smaller peak indicates a significant decrease of messenger RNA synthesis induced by the drug.

The discovery of polyadenine sequence of messenger RNA molecules was the basis for the development of specific methods for its isolation by affinity chromatography on cellulose coupled with deoxythymidine, which allowed interpretations regarding messenger RNA synthesis [6].

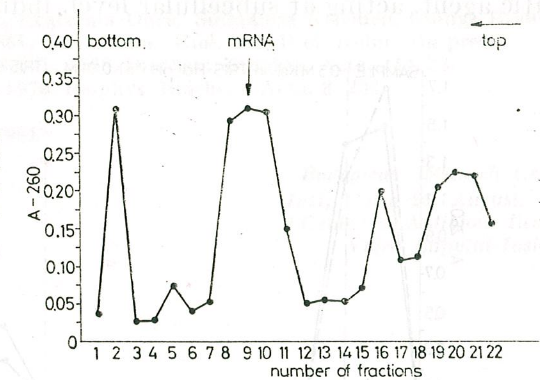

Fig. 1. — RNA profile of control HeLa cells analysed on 5—20% sucrose gradients, spun at 36,000 rpm at 2°C for 120 min.

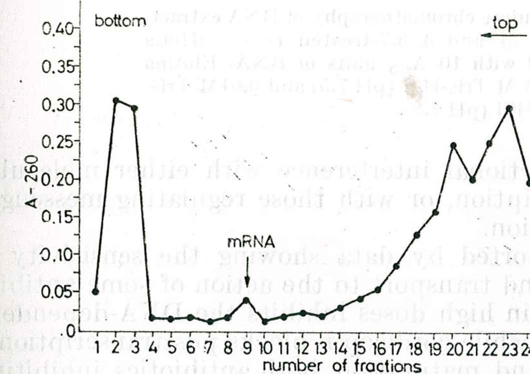

Fig. 2. — RNA profile of A 6.7-treated HeLa cells analysed on 5—20% sucrose gradients, spun at 36,000 rpm at 2°C for 120 min.

Fig. 3 shows the elution patterns obtained by affinity chromatography on oligo (dT) cellulose of the RNA samples extracted from control and A 6.7-treated cell cultures. The profile of RNA from controls is characterized by two distinct peaks. The first peak, representing excluded material, is usually due to ribosomal RNA which is eluted with high ionic concentration buffer from the column. Messenger RNA is retained by the oligo (dT) cellulose and is eluted with salt-free buffer as a second peak.

The elution profile of RNA extracted from cell cultures treated with the preparation A 6.7. showed the same characteristic peaks for both ribosomal and messenger RNA, but the latter had a lower amplitude indicating a decrease of messenger RNA by 50 per cent.

On this basis, it appears that the possible action of the preparation A 6.7 at molecular level is the inhibition of messenger RNA synthesis.

The synthesis of messenger RNA, template of protein synthesis, is a complex process which in eukaryotic cells takes place in several steps. A first step is transcription, which is a DNA-dependent process [7]. The second, posttranscriptional, is represented by adenylation and maturation of messenger RNA [1].

It may be stated that the antibiotic preparation A 6.7, a potential cytostatic agent, acting at subcellular level, induces the inhibition of mes-

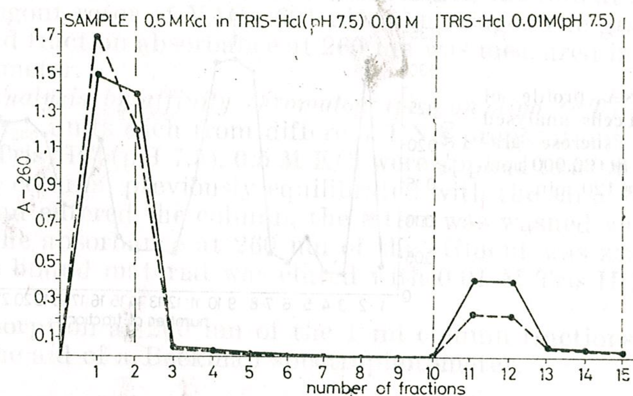

Fig. 3.— Oligo(dT) cellulose chromatography of RNA extracted from control (———) and A 6.7-treated (— — —)HeLa cells. Profiles obtained with 10 A_{260} units of RNA. Elution with 0.5 M KCl in 0.01 M Tris-HCl (pH 7.5) and 0.01M Tris-HCl (pH 7.5).

senger RNA synthesis by functional interference with either molecular mechanisms regulating transcription, or with those regulating messenger RNA adenylation and maturation.

This supposition is supported by data showing the sensitivity of messenger RNA biosynthesis and transport to the action of some antibiotics [3]. Thus, actinomycin D in high doses inhibits the DNA-dependent messenger RNA transcription, while cordicepin blocks posttranscriptional messenger RNA adenylation and maturation, both antibiotics inhibiting its synthesis by different mechanisms.

Additional investigations are needed to clarify the intimate mechanism by which this drug, studied by us, exerts its action at molecular level.

The mechanism of action suggested by us for the antibiotic preparation A 6.7 explains the inhibitory effect of this drug on HeLa cell culture development, evaluated on the basis of cellular protein synthesis registered in "in vitro" tests [10].

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A ZOOGEOGRAPHIC ANALYSIS OF THE SPECIES OF THE SUPERFAMILY PENTATOMIDAE, Reuter 1910, AS RECORDED IN ROMANIA

BY

I. ROȘCA

Correlation between similitude in the Heteroptera fauna of the superfamily Pentatomidae, Reuter, 1910, and geographic proximity or environmental conditions was carried out in nine zones of Europe, Africa and Asia.

In Romania, data concerning the Heteroptera are disparate and are included in faunal studies on limited areas [6, 7]; most authors give lists of all the Heteroptera captured in the investigated zone or only of the species of one family [1, 11], or even of the species in one genus [12]. Relatively few works characterize zoogeographically the heteroptera species [6, 7, 16, 17], while others discuss in a few lines the totality of the analysed fauna [11, 18].

MATERIAL AND METHOD

In order to perceive the kinship of the fauna of existing Heteroptera in Romania with that of other geographic zones, a zoogeographic analysis of the representatives of the superfamily Pentatomidae, Reuter, 1910 (the *Scutelleridae*, Leach 1815, *Pentatomidae* Leach, 1815 *Acanthosomatidae*, Stal 1865, *Cydnidae*, Billberg, 1820 and *Plataspidae*, Dallas 1851 families) from Romania [16, 17] and of a similar fauna in the Ukraine [14], Central Asia [15], Tien-Shan [13], Germany [23], Spain [2, 3, 4] Turkey [5], North-East Africa [8, 9, 10, 21], and North-West Africa [22] was carried out. Stichel's synthesis work [19] on the European Heteroptera completed the data concerning the distribution of the analysed species.

Proximity (kinship) of the superfamily, *Pentatomidae*, Reuter 1910, existing in the nine compared zones, was established by the use of the formula :

$$\rho_0 = \frac{\lambda \rho_g + \rho_{sp}}{\lambda + 1}$$

in which :

ρ_0 — index of fauna correlation and discrimination

ρ_g — index of genuses correlation and discrimination

ρ_{sp} — index of species correlation and discrimination.

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The correlation and discrimination indexes were calculated according to the Ekman formula, modified by Stugren and Rădulescu [20]

$$\rho = P(Ma - (Ma \cap Mb)] + P[Mb - (Ma \cap Mb)] - P(Ma \cap Mb)]$$

in which :

M^a — the totality of groups existing in the A area
M^b — the totality of groups existing in the B area
$Ma \cap Mb$ — the totality of groups common to both areas
P — the probability of multitudes.

RESULTS AND DISCUSSIONS

As may be seen in Table, 1, the number of species and genera of the superfamily Pentatomidae, Reuter 1910, is considerably higher in the southern zones of the analysed area; North-West Africa (71 genera, 187 species), Turkey (71 genera, 193 species), Spain (64 genera, 161 species).

Table 1

Number of the species and genera of the superfamily PENTATOMIDAE, Reuter 1910, recorded in different zones of the Palearctic region

Country or geographic zone	Fam. PLATASPIDAE		Fam. CYDNIDAE		Fam. SCUTELLERIDAE		Fam. PENTATOMIDAE		Fam. ACANTHOSOMATIDAE		Total	
	Sp.	Ge	Sp.	Ge.	Sp.	Ge.	Sp.	Ge	Sp.	Ge.	Sp.	Ge.
ROMANIA	22	1	20	10	11	6	67	35	6	4	106	56
UKRAINE	1	1	19	10	17	6	78	41	5	4	120	62
TIEN-SHAN	—	—	7	6	13	7	54	33	1	1	75	47
CENTRAL ASIA	2	1	38	12	40	14	177	57	3	2	260	86
TURKEY	3	1	26	11	32	7	130	50	2	2	193	71
NORTH-EAST AFRICA	1	1	24	10	22	6	69	36	—	—	116	53
SPAIN	1	1	30	12	18	6	111	14	1	1	161	64
GERMANY	1	1	16	8	11	5	52	28	7	4	87	46
NORTH-WEST AFRICA	2	1	44	18	31	10	110	42	—	—	187	71
Total number of species recorded in the Palearctic region	33		99		107		797*		37		1073	

* except the Urostilidae family

and particularly the arid region of Central Asia (86 genera, 260 species), as compared to the more north sited regions such as Romania (56 genera, 106 species), the Ukraine (62 genera, 120 species), Germany (46 genera, 106 species) or the zone of the high Tien-Shan mountains (47 genera, 75 species).

It has been found that a variation in the number of species and genera occurs particularly within the following families: the *Cydnidae* family, 6 genera with 7 species in Tien-Shan, as compared to 18 genera with 44 species in North-West Africa; the *Scutellaridae* family, 5 genera with 11 species in Germany, as compared to 14 genuses with 40 species in Central Asia and the *Pentatomidae* family, 28 genera with 52 species in Germany as compared to 57 genera with 177 species in Central Asia. The *Plataspidae* and *Acanthosomatidae* families are feebly represented in the Heteroptera fauna of the compared zones.

The ρ_0 synthetic index most truthfully reflects (Fig. 1) the real faunal kinship conditions of the superfamily *Pentatomidae* Reuter 1910, in the nine analysed areas.

The Heteroptera fauna of all the analysed zones has common elements; from a faunal viewpoint there is no zone completely different from another.

Faunal discrimination in the superfamily *Pentatomidae* Reuter 1910, is highest between the fauna of Germany and of North-East Africa ($\rho_0 = +0.53$) and faunal correlation is highest ($\rho_0 = -0.61$) when comparing Romanian fauna with the Ukraine one.

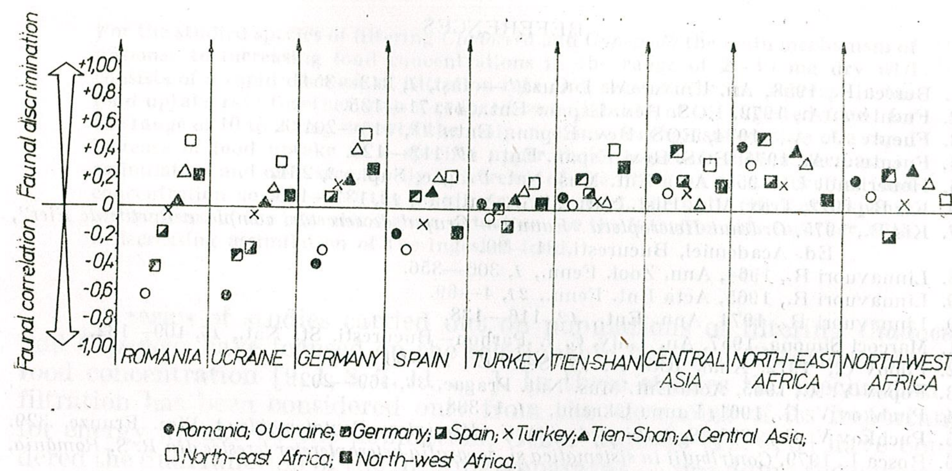

Fig. 1. — Plotting of ρ_0 (the index of faunal correlation and discrimination).

As for the faunal kinship of the five analysed families, Romania is, in succession, faunistically related to the Ukraine ($\rho_0 = -0.61$), to Germany ($\rho_0 = -0.41$) and to Spain ($\rho_0 = -0.18$); the Ukraine has a fauna similar to that of Romania ($\rho_0 = -0.61$), of Germany ($\rho_0 = -0.32$) and of Spain ($\rho_0 = -0.30$); Spain's fauna is similar to that of North-West Africa ($\rho_0 = -0.19$); Turkey has faunal similitudes with all the other compared zones ($\rho_0 = -0.12$; $+0.19$); the other analysed areas have common faunal elements, but elements specific to the zone predominate. North-East Africa occupies a somewhat special position, for here — like in Central Asia — the eremial species predominate; an increased influence of the neighbouring Ethiopian region is simultaneously felt.

The faunal similitude may be correlated with geographic proximity (Romania — the Ukraine, Spain — North-West Africa), or with environmental conditions which determine faunal similitudes between zones sited at higher latitudes (Germany, Romania and the Ukraine) and eventually similitudes between these and the fauna existing in the Tien-Shan mountainous zone; between the arrid zones generally sited at a more reduced latitude (North-East Africa, Central Asia, Turkey).

CONCLUSIONS

1. There is a correlation between similitude in the Heteroptera fauna of the superfamilly Pentatomidae, Reuter 1910, and geographic proximity or environmental conditions.

2. Considering the similitude of the fauna superfamily in nine zones of Europe, Africa and Asia, faunal discrimination does not exceed the ρ_0 estimate of + 0.53 when comparing Germany's fauna to that of North-East Africa, while faunal correlation is highest ($\rho_0 = -0.61$) between Romania's and the Ukraine's fauna.

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THE RELATIONSHIP BETWEEN FILTERING ZOOPLANKTON AND PHYTOPLANKTON

II. The influence of algae concentration upon the filtration rate

BY

ANGHELUŢĂ VĂDINEANU and RALUCA MUNTEAN

For the studied species of filtering *Cladocera* and *Copepoda* the main mechanism of response to increasing food concentrations in the range of 2—10 mg dry wt/l consists of a rapid decrease of filtration rate and, secondarily an increase in the food uptake rate together with a reduced assimilability of the ingested food; for the range of 10 to 20 mg dry wt/l the main response mechanism consists of a rapid increase of food uptake to a specific upper limit, together with a decrease in assimilation and only secondarily a decrease of the filtration rate. Once the food concentration goes beyond the 20 mg dry wt/l level, the only efficient response mechanisms of the organisms are a reduction of the filtration rate together with a decreasing assimilation of the ingested food.

A series of studies carried out on populations of filtering *Cladocera* and *Copepods* have focused on the relationship between filtration rate and food concentration [9, 1, 8, 7, 11 . . .]. In these studies the mechanism of filtration has been considered only from the viewpoint of its importance for energy uptake by the population. Other workers [12, 6, 5] have considered the dual function of the filtering apparatus — providing the necessary energy intake and the necessary oxygen — trying to explain the relationship between the filtration rate, the amount of food in suspension and the respiration of the animals. According to these workers, for a food concentration of less than 0.2 mg dry wt/l the filtration rate reaches a constant maximum but becomes dependent on the food concentration at values ranging from 0.2—20 mg dry wt/l. Ivanova (1970), considering that only part of the water soluble oxygen can be used at the level of the filtering apparatus — the highest efficiency being around 30—35% —, appreciates that as the food concentration increases the filtration rate decreases down to a minimum level which remains constant. This lower limit of the filtration rate, corresponding to a food concentration of about 20 mg dry wt/l, seems to be determined by the oxygen requirement. Under these conditions, although the food concentration exceeds 20 mg dry wt/l, the only response of the organisms seems to be a decrease in assimilability.

Our work on certain species of *Cladocera (Daphnia magna, Daphnia pulex, Simocephalus vetulus)* and one species of copepod — *Eudiaptomus gracilis* — is an attempt at determining the effects of varying food concentration between 0.2—20 mg dry wt/l and 20—50 mg dry wt/l on filtration rate.

REV. ROUM. BIOL.—BIOL. ANIM., TOME 28, N° 1, P. 59—63, BUCAREST, 1983

MATERIALS AND METHODS

As a source of food for the animals, cultured *Chlorella* and *Scenedesmus* algae were used as the dimensions of these algae are within the range of particles that can be filtered by them. The filtration rate was determined for five algae concentrations from 0.3 to 50 mg dry wt/l, at four temperatures between 8–25°C and four size classes between 3–60 µg dry wt for *Cladocera* and 1–20 µg dry wt for copepods. Groups of individuals from the same size-class were being used to the experimental conditions for 1–2 days, then maintaind for 2–3 hours in water filtered through a 0.45µ membrane filter and finally transferred to the desired experimental set up. The samples so prepared were kept in the dark for a period of 30 to 60 minutes. The length of the experimental period was chosen on the basis of reports in the literature showing that after about one hour undigested food is being eliminated [12, 10]. The initial (Co) and final (Ct) algae concentrations were determined from the recorded absorption at 670 nm plotted on a standard curve established with known algae concentration. The filtration rate was worked out according to Gould [5] and expressed as ml/ind/24h and as ml/mg dry wt/h.

RESULTS AND DISCUSSIONS

As can be noticed from Fig. 1A, the filtration rate of an animal of 8 µg dry wt belonging to the *Cladocera* species studied, decreases considerably with an increase of food concentration in the range of 0.5–15 mg dry

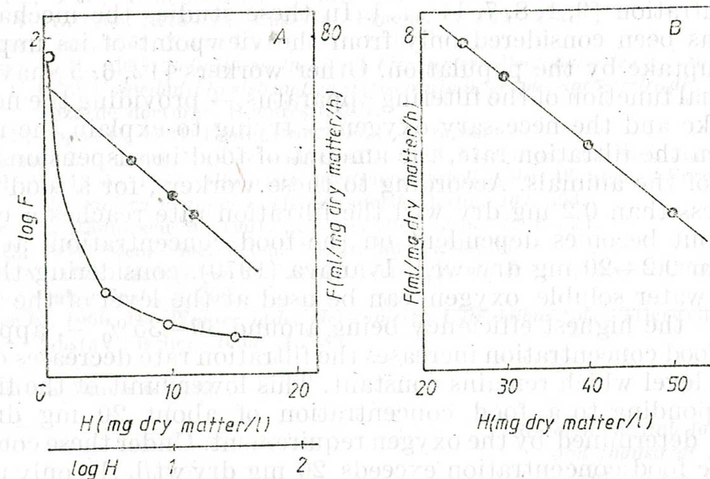

Fig. 1. — The filtration rate as a function of food concentrations in *Daphnia magna*

W = 8 µg dry matter; t = 20°C
A — (H ⊂ 0.5 – 30 mg dry matter/l)
B — (H> 20 mg dry matter/l)

wt/l, the dependence of the filtration rate assumes a typical curve which becomes a straight line when transformed in logarithms. The filtration rate determined for the 15 mg dry wt/l algae concentration represents only

10% of the value recorded for a 0.5 mg dry wt/l algae concentration. It is worth mentioning that after they get used to an increased food concentration in the range of 20–50 mg dry wt/l, the specimens belonging to the species studied reduce the filtration rate from 9 to 3.6 ml/mg dyr wt/h which means a 60% decrease.

The reduction of the filtration rate following an increase of food concentration above the 20 mg dry wt/l level follows a straight line (Fig. 1B) which refutes the hypothesis according to which there is a constant minimal

Table 1

The mathematical expressions obtained from the unitary analysis of the recorded data for the *Cladocera* species and *E. gracilis* as well as the values of the F test of correlation (W-mg dry weight; H-food concentration in mg dry wt/l; F = ml/mg dry wt/h)

Regression models	R^2	F
Cladocera		
1) H <20 mg log F=1.51–0.62logH– –0.0912logW	0.94	43**
2) H>20 mg F = 11.88–0.168 H	0.93	27**
E. gracilis		
1) H<20 mg log F=1.43–0.415 log H– –0.089logW	0.93	18**
2) H>20 mg F = 12.08–0.158 H	0.96	15.7**

** Significant for the 99% confidence limits.

value of the filtration rate imposed by the need to supply the necesary oxygen in order to release the maintenance energy. Using the mean values of the filtration rate determined for groups of individuals of varying dimensions and for different values of food concentration in the mentioned ranges, regression models have been established; filtration rates can be warked out on the basis of these models (Table 1). Since in conditions of excess food (H > 20 mg dry wt/l) the filtration rate undergoes proportionally a very small modification as compared to the size of specimens (r^2 = 0.02), the filtration rate is expressed only as a function of food concentration.

In *Eudiaptomus gracilis*, a similar kind of relationship has been found between food concentration in the medium and filtration rate like in the species of *Cladocera* — a curve for food concentrations of of 0.5 to 20 mg dry wt/l and a straight line for 20 to 50 mg dry wt/l (Fig. 2A and 2B). As compared with the studied species of *Cladocera*, *Eudiaptomus gracilis* responds differently to similar increases in food concentration. For individuals of similar sizes, and at the same food concentrations, the filtration rate is smaller in *E. gracilis* than in the studied *Cladocera* and moreover, the filtration rate is influenced to a lesser degree in *E. gracilis* than in the *Cladocera* when increasing the food concentration.

For instance, in individuals of a mean dry weight of 8 µg kept at a food concentration of 0.5 mg dry wt/l, the filtration rate is 55 ml/mg dry wt/h in *E. gracilis* as compared with 77 ml/mg dry wt/h in *Cladocera* of

similar size and kept in similar conditions. Similarly, the filtration rate of *E. gracilis* is affected to a lesser degree by increased food concentration, the recorded value at a food concentration of 15 mg dry wt/l was 24 % of the value recorded at 0.5 mg dry wt/l as compared with only 10 % for the

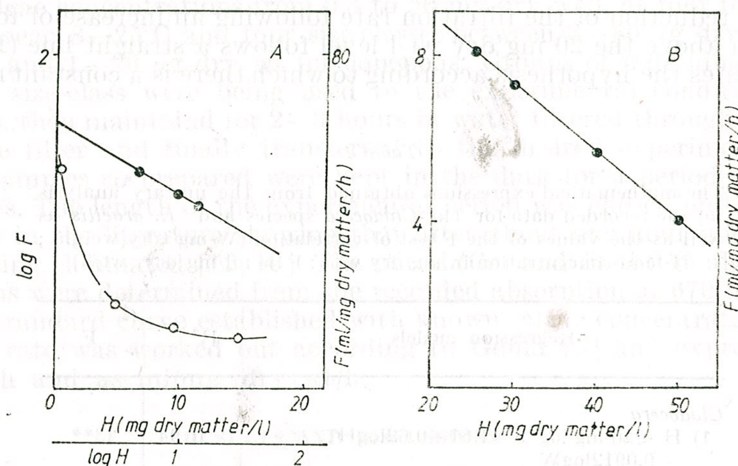

Fig. 2. — The filtration rate as a function of food concentrations in *Eudiaptomus gracilis*

$W = 12\,\mu g$ dry matter ; $t = 20°C$
A – (H ⊂ 0.5 – 20 mg dry matter/l)
B – (H > 20 mg dry matter/l)

species of *Cladocera* in similar conditions. The filtration rate for an increase in the food within the range of 20—50 mg dry wt/l shows a reduction of only 49 % in *E. gracilis* as compared with 60 % reduction recorded for *Cladocera*. As can be seen from these results the filtration rate does not tend to reach a minimum value, that is maintained over a large range of food concentrations in the medium. In conditions of excess food it appears to be a strong stressing factor for the filtrating organisms, in the case of a constant filtration rate, in order to ensure the necessary oxygen and it would mean that the food uptake rate would have to increase proportionally with the food concentration. This onesided response is not adequate as it enhances the stressing effect of excess food by exceeding the food uptake and food digestion capacity of the species. The reduction of the filtration rate below the minimum value that ensures the necessary oxygen for the filtrating species of *Cladocera* and *Copepoda* studied by us in conditions of excess food is not unexplainable, on the contrary, it is an important argument supporting the idea that natural populations do transform, under pressure from the environmental factors, evolving multiple possible responses, one or another of these evolved mechanisms having a dominant role for certain values of the environmental factors. We consider, therefore, that in the studied species, the main mechanism of response to an increasing food concentration in the range of 3 to 10 mg dry wt/l is a rapid decrease of the filtration rate and, secondarily, an increased food up-

take combined with a reduction in the assimilation rate. On the other hand, if the food concentration ranges between 10 to 20 mg dry wt/l the organisms will respond mainly by rapidly increasing the food uptake and, at the same time, reducing assimilability and only secondarily by decreasing the filtration rate. For food concentrations above 20 mg dry wt/l, since the maximum capacity for food ingestion had been reached other mechanisms such as decreasing filtration rate or assimilability (u^{-1}) will have to be used even if it will lead to hypoxia which in turn will profoundly influence the energy flow (Vădineanu 1980*un*published). The filtration rate expressed in ml/ind/24 h fluctuates within wide limits. For instance in individuals of *Cladocera* with a mean dry weight of 8 μg, the maximum volume of water filtered at a concentration of 0.5 mg dry wt/l is 15 ml while for a food concentration of 50 mg dry wt/l it is only 0.73 ml. Individuals of *E. gracilis* with the same mean dry weight and at similar food concentration conditions showed a maximum filtration rate of 10.8 ml while the minimum was 0.83 ml. A high filtration rate of 10—36 ml/ind/24 h has been reported for large individuals with a mean dry weight of 100 μg belonging to the genus *Calanus* which have been grown in a medium with a food concentration of 5—6 mg dry wt/l [2, 3]. For individuals of *D. pulex* with a mean dry weight of around 15 μg grown at a food concentration of 4 mg dry wt/l, a filtration rate of 4—5 ml/ind/24 h [8, 10] has been reported.

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THE WORKING SESSION OF THE REPRESENTATIVES OF THE NATIONAL COMMITTEES FROM SOCIALIST COUNTRIES FOR THE INTERNATIONAL UNION OF BIOLOGICAL SCIENCES (IUBS), BUCHAREST, 22–24 JUNE 1982

After the meeting held on June 1981 in Budapest, this 6th working session was organized on 22–24 June in Bucharest, under the sponsorship of the Academy of the Socialist Republic of Romania. The members of the Romanian National Committee were pleased to welcome the following guests: acad. prof. V. Landa from Czechoslovakia, prof. J. Salanki and dr. P. Biro from Hungary, prof. A. Urbanek from Poland, acad. prof. M. S. Ghilarov and dr. S. G. Vassetzky from USSR. The agenda of this session included such topics as the participation of delegations from the socialist countries to the XXI IUBS General Assembly in Ottawa, Canada, August 1982, the election of the officers of the new Executive Committee of the IUBS and the problems of the future structure of the IUBS divisions. There were also discussed proposals for different research projects of general or regional interest to be carried out within the framework of IUBS, i. e. the establishment of a group of systematic and evolutionary biology, at the request of the Polish National Committee and completion of the necessary research for drawing up the vegetation map of Europe. Plans were submitted for the organization in 1984, in USSR, of a Symposium on the early stage of ontogeny and of a European conference on cell biology in Hungary, in 1986. Wishes were expressed for a closer cooperation between the scientific institutions of the socialist countries, especially in the following fields: publication of scientific journals by an international editorial board, production of scientific equipment and chemicals, rearing of genetically controlled laboratory animals. Visits were paid to the Institute of Cellular Biology and Pathology, the Institute of Biological Sciences, the "Fundulea" Research Institute for Cereals and Technical Plants and the "Gr. Antipa" Natural History Museum, or others, optionally.

During all these contacts the stringent need appeared for a permanent climate of peace absolutely necessary to assure human welfare and the progress of scientific work through the cooperation of world biologists within IUBS in relation with UNESCO and ICSU to firmly act against the threats of armed conflagrations and especially of a nuclear war.

The following opening address by acad. prof. Radu Codreanu, on behalf of the Romanian National Committee for IUBS, summarizes the scientific trends and the pacific aims of biologists from socialist countries who gathered in Bucharest.

REV. ROUM. BIOL. – BIOL. ANIM., TOME 28, N° 1, P. 65–67, BUCAREST, 1983

Dear Colleagues,

It is a great honour and pleasure for the Academy of the Socialist Republic of Romania to offer its hospitality to the present working session of the prominent representatives of the National Committees for the International Union of Biological Sciences, IUBS, from socialist countries. We are happy to express the warmest welcome to Bucharest to our guests from Czechoslovakia, Hungary, Poland, the Soviet Union, and we regret the absence of our colleagues, biologists from Bulgaria, Yugoslavia and the German Democratic Republic.

The goal of the International Union of Biological Sciences is the better coordination and cooperation between the activities of biological organizations from different countries. Since geographic proximity raises many common problems for biological research, periodic exchanges of our results proved to be very useful.

Modern biology is highly diversified, including topics on molecular processes in the living cells as well as questions of the dynamics of the ecosystems of the biosphere. This multiplicity of branches of biological knowledge should be reflected more and more adequately so as to be employed by the IUBS divisions in bringing to the fore the vital importance of advances in fundamental and applied biology for the development of human society.

The use of physical and chemical technologies was certainly decisive for the revolutionary progress of biology in this century. But, it must not be forgotten that all scientific activities are due only to the creative powers of the human brain in our capacity of rationally acting beings. Therefore, the field of biological implications is much more extensive and acquires a basic significance during our endeavours to clear up the major factors of interest in human evolution. Indeed, biology is able to supply us the theoretical elements for the understanding of man's place within the evolutionary history of life on earth and also to solve, by means of its applied disciplines, many important practical problems, such as man's health, the preservation of the natural equilibrium, the increase of biological productivity confronted with the demographic overflow.

The present complexity of the difficult relationship between mankind and its environment runs the risk of heavy deterioration as a result of the energy crisis and the nuclear arms race. Let us hope that the strong movement for peace recently started throughout the world and which has long and firmly been promoted by the President of Romania, Nicolae Ceaușescu, shall prove successful and efficient.

The setting up, in the last year, after the 16th International Congress of the History of Science held in Bucharest, of an official body called "Scientists and Peace", presided over by Academician Elena Ceaușescu, D. Chem. Eng., first-vice-prime minister of the Government of the Socialist Republic of Romania, President of the National Council for Science and Technology, gives an optimistic suggestion that the International Union of Biological Sciences may also constitute an important background for the defence of peace since biologists are best qualified to appreciate the wonderful unicity of life and the human species on our planet.

The evolution of the biosphere was a multi-millenary way that led to the emergence of human consciousness which has the unique privilege of reflecting universal reality. Without mankind, there will be nothing left to attest the eternal organizing movement of matter, or our splendid cultural values. It is therefore our most justified common duty to preserve humanity and the biosphere against the radical destructions of a nuclear war.

The trends for scientific cooperation of the International Union of Biological Sciences are both pacific and transdisciplinary.

With all this in mind, we wish our participants to broach with fruitful achievements the topics listed on the agenda of our three-day meeting and believe that the following report of acad. M. S. Ghilarov on the last session of the IUBS Executive Committee, Paris, April 1982, will introduce us into the most topical problems before the next General Assembly to be held in August 22–27, in Ottawa, Canada.

LE CENTENAIRE DU LABORATOIRE ARAGO À BANYULS-SUR-MER, FRANCE

Le Laboratoire Arago, centre d'océanographie biologique d'une haute renommée internationale situé aux confins des Pyrénées Orientales dans la Méditerranée et dépendant actuellement de l'Université Pierre et Marie Curie à Paris vient de fêter son centenaire par de prestigieuses manifestations déroulées pendant les journées du 12 et 13 octobre 1982. En dehors des témoignages historiques des personnalités françaises, telles les professeurs P. P. Grassé, P. Drach, A. Guille, P. Bougis, Cl. Delmare Deboutteville, J. Théodoridès, J. Soyer, plusieurs savants étrangers ont tenu à évoquer les recherches effectuées par leurs compatriotes grâce aux conditions favorables offertes dans le cadre du Laboratoire Arago. C'est ainsi que les professeurs P. Tardent (Zurich), B. Battaglia (Italie), R. Reinboth (Mayence) et W. Geilenkirchen (Utrecht) ont rappelé les liens unissant les biologistes de leurs pays aux activités scientifiques du Laboratoire Arago.

Pour la biologie roumaine, le centenaire du Laboratoire Arago revêt une signification toute particulière en raison de la brillante carrière scientifique qu'Emile Racovitza y déploya pendant plus de trente ans, y étant attaché comme sous-directeur de 1900 à 1920, période de sa collaboration extrêmement féconde avec Georges Pruvot, René Jeannel, Louis Fage et bien d'autres éminents zoologistes français et étrangers. C'est ce qui justifie l'exposé du dr. Mihai Băcescu sous le titre : « Emile Racovitza, un nom roumain entré dans l'histoire du Laboratoire Arago », qu'il fit aux journées du Centenaire, où il représenta notre pays en même temps que dr. Traian Orghidan de la part des spéléologistes de Roumanie.

Grâce à l'appui de notre Etat, depuis 1965, un buste particulièrement expressif dû au sculpteur Gh. Anghel perpétue la mémoire d'Emile Racovitza dans l'enceinte du Laboratoire Arago et le compte rendu de son inauguration officielle se trouve largement consigné dans le périodique français « Vie et Milieu », tome XIX, 1968, fasc. 2C, p. 503—528, où l'œuvre mémorable de Racovitza à Banyuls-sur-Mer est magnifiquement retracée.

Pour marquer l'ancienneté de plus d'un siècle des relations des biologistes avec les maîtres de la zoologie française, notamment H. de Lacaze-Duthiers, l'illustre fondateur du Laboratoire Arago, et leur importance pour le développement des sciences biologiques en Roumanie, nous reproduisons ci-après le texte d'hommages adressé à l'occasion du Centenaire de la Station de Banyuls-sur-Mer au nom de la Section des sciences biologiques de l'Académie de la République Socialiste de Roumanie.

À LA DIRECTION DU LABORATOIRE ARAGO DE LA PART DE LA SECTION DES SCIENCES BIOLOGIQUES DE L'ACADÉMIE DE LA RÉPUBLIQUE SOCIALISTE DE ROUMANIE

L'anniversaire du Centenaire de la fondation du Laboratoire Arago à Banyuls-sur-Mer suscite le plus haut intérêt parmi tous les biologistes roumains vu les répercussions durables que l'œuvre grandiose des éminentes personnalités de Henri de Lacaze-Duthiers et d'Emile G. Racovitza ont eues sur le développement des relations scientifiques franco-roumaines et les progrès des sciences biologiques en Roumanie. Mû par la foi ardente d'un apôtre, Lacaze-Duthiers fut un chef d'école d'une envergure inégalable, ayant promu la biologie marine en France par la triple création de la Station Biologique de Roscoff (1872), des Archives de Zoologie expérimentale et générale (1872) et du Laboratoire Arago à Banyuls (1882), qui enrichirent non seulement la France, mais devinrent bientôt des centres de recherche d'ordre international. C'est

ce qui explique qu'avant même l'ouverture du Laboratoire Arago, les Archives de Zoologie font paraître les thèses de doctorat de deux Roumains, celle de Léon C. Cosmovici sur les *Glandes génitales et les organes segmentaires des Annélides Polychètes* (1879) et celle d'Alexandre N. Vitzou sur *La structure et la formation des téguments chez les Crustacés Décapodes* (1882), dont le temps n'a fait qu'augmenter la valeur.

La remarquable thèse d'Emile Racovitza sur *Le Lobe céphalique et l'encéphale des Annélides Polychètes* (1896) leur fait suite et à l'issue de sa retentissante participation à l'*Expédition antarctique Belge* (1897—1899), toute sa conduite scientifique lui attire à tel point la confiance de Lacaze-Duthiers qu'à partir de 1900, à côté de Georges Pruvot, Racovitza accueillit le principal héritage de leur Maître : la codirection des Archives de Zoologie et le rang de sous-directeur du Laboratoire Arago. Le Volume Jubilaire publié lors du Cinquantenaire du Laboratoire de Banyuls (1932) dans les Archives de Zoologie (1937) a mis en relief le puissant apport de Racovitza aux activités de ce Laboratoire et Racovitza lui-même y prit part avec joie, en revenant de l'Institut de Spéologie de Cluj, qu'il avait fondé en 1920 en Roumanie. C'est toujours sous les auspices du Laboratoire Arago que fut scellée aux environs de 1904 la féconde collaboration d'Emile Racovitza avec René Jeannel, marquant l'avènement de la *Biospeologica*, autrement dit le démarrage du considérable mouvement qu'est la Spéléologie moderne. Ce sont ces raisons qui ont soutenu notre initiative de mener à bien comme dons de l'État Roumain l'installation des bustes d'Emile Racovitza au Laboratoire de Banyules (1965) et celui de René Jeannel au Muséum National d'Histoire Naturelle à Paris (1969).

Et il est temps de nous rendre compte que de l'effort de ces deux grands savants à comprendre l'évolution des lignées souterraines, est véritablement née la « nouvelle Systématique évolutive », quelques décennies plus tôt qu'elle est couramment attribuée aux ouvrages de J. Huxley, G. G. Simpson et E. Mayr (1942). Par rapport à ces derniers, les travaux de Racovitza et Jeannel n'ont pas seulement anticipé l'importance de la spéciation géographique et des modalités de l'isolement dans la genèse des espèces, mais ils ont eu le mérite de postuler la signification géonémique et historique de la Systématique, conçue comme base inaliénable de l'évolutionnisme. N'omettons non plus que le fidèle compagnon des deux grands classiques de la Spéléologie, Pierre Alfred Chappuis, c'est dans les parages de Banyuls qu'il a accompli ses dernières recherches sur la faune interstitielle avec le futur professeur Claude Delmare-Deboutteville. Un ému hommage est également adressé à la mémoire des personnalités qui à la suite de G. Pruvot ont assumé la direction du Laboratoire Arago : les professeurs Octave Duboscq, Edouard Chatton, Georges Petit et en dehors d'eux, à celle de Louis Fage.

N'insistons pas davantage sur la justification du message que les biologistes membres de L'Académie Roumaine désirent apporter de la manière la plus chaleureuse à la célébration d'un événement scientifique international aussi important que le centenaire du Laboratoire Arago. Que l'on veuille bien agréer nos vœux les plus sincères pour sa prospérité continuelle à l'avenir et considérer le Laboratoire Arago comme un symbole perpétuel de la coopération scientifique franco-roumaine.

Académicien 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 les articles, notes et comptes rendus dactylographiés à double interligne (31 lignes par page) en deux exemplaires.

La bibliographie, les tablaux 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 dans les graphiques sera évitée. Les références bibliographiques, citées par ordre alphabétique 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 aux 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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