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NEW BENTHIC MYSIDS FROM THE LITTORAL WATERS OF KENYA: *MYSIDOPSIS KENYANA* n.sp. AND *NOUVELIA NATALENSIS MOMBASAE* n.g., n.sp.

BY

MIHAI BĂCESCU and ELENA VASILESCU

The authors describe, from the shallow, littoral waters (0.5—1 m.) of Kenya, near Mombasa, a new species of *Mysidopsis* — *M. kenyana* n.sp. — and a new subspecies belonging to the *Tenagomysis* group, for which a special genus was instituted, *Nouvelia* n.g. — namely *Nouvelia natalensis mombasae* n.ssp. Zoogeographical comments are given.

One of the authors (El. Vasilescu) had the opportunity to dredge in the shallow waters in front of Mombasa (Kenya), capturing an abundant material of crustaceans, especially amphipods, Cumacea and mysids, among which numerous species new for science. We give hereafter the description of two new species of mysids, namely:

Mysidopsis kenyana n.sp.

(Fig. 1)

Carapace anteriorly ends by a large, bulky rounded rostrum (Fig. 1 *B*) which reaches the middle of the A_1 median segment. Anterolateral extremities wide, ± circular (Fig. 1 *C*) narrowing at the inferior portion, towards the limit of cervical notch, where it covers a wide ventilation aperture. This structure is strikingly similar to that of *Paramysis baeri bispinosa*, in which the long plumose setae of maxilla II activate there. It is, of course, a convergence phenomenon determined by a certain psammobiontic way of life.

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Pleon compact, last segment slightly shorter than telson.

Antennae stout, short, with a huge lobe in the ♂, longer than its supporting segment. A_2 with a shorter basis than A_1 and a scale distinctly exceeding A_1 level. Scale ± oval (Fig. 1 *A*), approximately thrice as long as wide, with a minute apical segment (Fig. 1 *A*) and a small spine on its sympodite.

Eyes globulous, perfectly circular in profile view, exceeding carapace margins by at least the half of their cornea thickness; cornea, brown-golden, represents almost 1/2 of the eye; peduncle suddenly narrows towards the ocular commissure. Labrum with rostral portion obtuse, almost round, spineless. Mandibular palp wide, its basal segment with a strong "heel", terminal segment hardly twice as long as broad; masticatory portion of mandible narrow, reduced.

Maxilliped II with apical portion of distal segment broad, provided with 4 strong spines (Fig. 1 *D*).

Peraeopods stout (Fig. 1 *E* and *G*), tarsus 3-segmented (Fig. 1 *F*) in pairs I—IV and bi-segmented in last pair (Fig. 2 *G*), slightly smaller than the others. Dactylus not even, but with eminences at the inferior portion, a feature specific to *M. kenyana*. Exopodites without spinules on the bases. In one ♀, last peraeopod in concrescence with the large oostega, reduced to a stump (Fig. 1 *H*); hadn't it been present in both legs, we might have said an appendage in process of regeneration.

Pleopods unilamellar in the ♀♀, with basis and rami well developed in ♂♂. Dimorphism weakly marked. Exopodite, dimorphic, has a single terminal seta and is somewhat longer than endopodite (Fig. 1 *I*).

Telson — linguiform (Fig. 1 *J*) both in ♂♂ and ♀♀ with approx. 13—14 lateral spines ± equal, with or without a small median hiatus and 2 strong apical spines: only exceptionally (anomaly) these apical spines are not symmetric (Fig. 1 *J*). A slight dimorphism in the telson, i.e. subapical spines longer (1/3 of the apical ones) in ♀♀ (Fig. 1 *K*).

Uropods, as broad thin plates; endopodite ending at the level of the apical spines of telson, armed by 5—6 minute spines in the statocyst portion (Fig. 1 *L*).

Colour. *Mysidopsis kenyana* impresses by its dark brown colour almost blackish. Not only the cornea, but also the buccal pieces, A_1 lobe in ♂♂, peraeopods, sternal face, abdomen, telson and uropods, in their proximal 3/4 are brown. Only the male pleopods rami, partially, and the pleopods lamellae in females are translucent. Oostegites also intensely brown.

Material examined, locality: 4 ♀♀ marsupiphore, 5 ♂♂ ad. + 15 juv. Kenya, Mombasa beach, by net in the waters of the inferior tidal limit (approx. 1 m.). Holotype ♂, Coll. of "Gr. Antipa" Museum, no. 222; allotype ♀, ditto, no. 222 a; 2 paratypes, ditto, no. 223.

Ecology, benthic species, adapted to life among brown algae, wherefrom its brown colour.

To the *M. kenyana* association belong, as main accompanying forms, in the first place, a *Gastrosaccus* (*Haplostylus*) n. sp., then *Siriella brevicaudata*, *Nouvelia natalensis kenyana*, *Exosphaeroma* sp. and hundreds of *Cumopsis* n. sp.

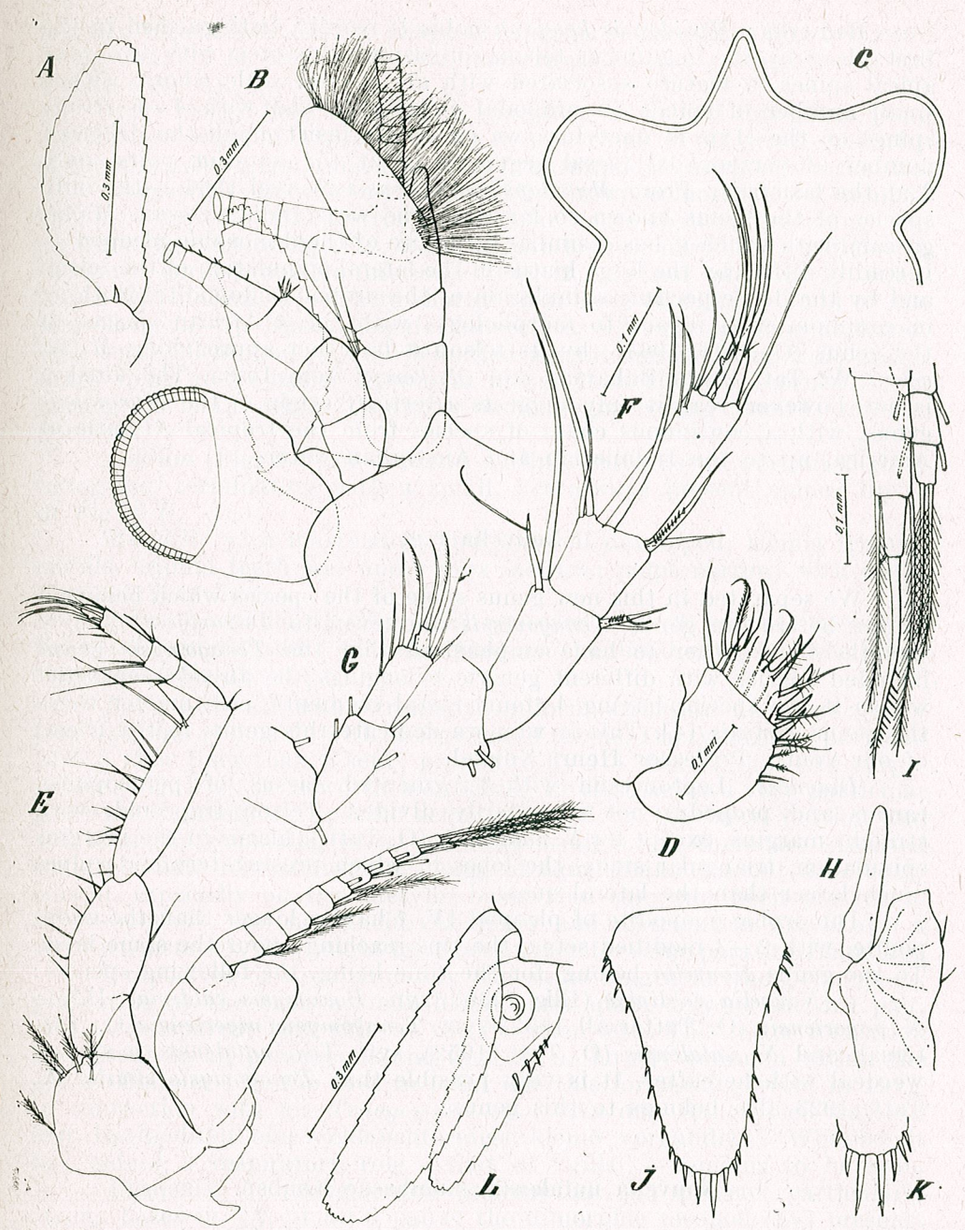

Fig. 1. — *Mysidopsis kenyana* n.sp. *A*, scale A_2 ♀; *B*, cephalic portion (top view) (♂); *C*, rostral portion of a ♀ carapace; *D*, distal segments of mxp. I (♀); *E*, peraeopod I; *F*, its "tarsus" magnified; *G*, peraeopod V ♂ and *H*, peraeopod V ♀, anomaly (in process of regeneration ?); *I*, distal portion of pleopod IV rami (♂) (dimorphic); *J*, telson ♂ with asymmetric apical spines; *K*, telson end in a ♀; *L*, uropods, ♀; fig. *L* scale is also valid for fig. *B*, *E*, *J*, *K* and *H* (orig.).

Remarks : *Mysidopsis kenyana* nobis is readily distinguished in the first place by its linguiform telson, oligospine provided with two long apical spines, a feature associated with an A_2 oval scale, short, with a small number of spines on uropodal endopodite and with 4—5 strong spines on the Mxp. II dactylus; we no longer insist upon the different number of peraeopodal tarsal segments (3 at the anterior pairs, only 2 at the last pair). From *Mysidopsis hellvilensis* Nouvel 1964 — the only species of the genus known so far from the west Indian Ocean (Madagascar), with which it has a similar structure of the dimorphic pleopod — it readily differs by the large hiatus in the lateral spinulation of the telson and by the dense pectinic spinulation of the uropod endopodite. Nothing in common, with regard to morphology, with the 4 African species of the genus (O. Tatt. 1964); by its telson it is rather approaching *M. bigelavi* W. Tatt. from Bahamas and *M. kempi* from India. The kinship is not, however, real, it only suggests a tertiary origin of the *Mysidopsis* genus, with a continuous chain of species from the tropical Atlantic of America, up to the Indonesian and Australian waters.

Nouvelia n.g.

We separated in this new genus some of the species which belonged to the heteroclite genus *Tenagomysis* and even to *Doxomysis*. Nouvel was the first author to have emphasized that the *Tenagomysis* genus included species with different generic belongings; he already separated within it the species having 4 (four) tarsal segments, creating for these the genus *Iimysis* [4]. This is why we dedicate this genus to the friend of our youth, Professor Henri Nouvel.

Diagnose. Leptomysini with 3-segmented tarsus of peraeopods; carpus and propodus not secondarily divided. Telson trapezoid, with straight margins, except for *N. natalensis* (O. Tatt.), hiatus in the margins spinulation, triangular sinus, the lobes of which present terminal spines much larger than the lateral ones.

Dimorphic exopodite of pleopod IV ♂ hardly longer than the endopodite, with 2—3 modified setae, the tips reaching about the same level. To the genus *Nouvelia* belong, for the time being, the following species :

— *Nouvelia valdiviae* (Illig 1930) (syn. *Doxomysis valdiviae* Illig); *N. nigeriensis* (O. Tattersall 1957) syn. *Tenagomysis nigeriensis* O. Tattersall and *N. natalensis* (O. Tatt. 1952), syn. *Ten. natalensis*, a species we deal with hereafter. It is very possible that *Tenagomysis similis* W. Tatt. 1923 also belongs to this genus.

Nouvelia natalensis mombasae n.subsp.

(Fig. 2)

The sspecies *Nouvelia natalensis* was described on the basis of a single specimen, male, non-adult and broken, lacking, among other pieces, just the pleopod IV, dimorphic. Hence, description is not complete, as

it doesn't include either the shape of labrum, or the shape and armature of the maxilla II palp, both first rate criteria in the systematics of the *Tenagomysis* l.s. genus.

If we assign here the Kenyan specimens — first of this genus along the Eastern coast of Africa — we do it on account of the great similarity of the carapace rostral projection and of the uropodal endopodite armature.

Description. Benthic mysids, vividly brown coloured, 6—7 mm. in length. Carapace with a strong triangular acute rostrum, reaching the medium segment of A_1 (Fig. 2 *A*); antero-lateral margins ("shoulders"), suddenly curved postero-inferiorly (Fig. 2 *A*). Last pleonite of equal length with telson. A_1 slightly shorter than scale of A_2 in ♀♀ and at the same level in ♂♂. Scale elongate, lanceolate, 5.3 times as long as broad, with setae all around, a well delimited apical segment (Fig. 2 *B*) and a sharp spine at its basis. Eyes oval rather than elongate, cornea representing 1/3 of the whole; peduncle suddenly narrowing towards anterior portion.

Labrum (Fig. 2 *C*) of ± round outline, with rostral portion slightly triangular, terminating with a small, yet always present spine; lip as in Fig. 2 *D*.

Mandible with masticatory portion well developed, lacinia triphid, mobile, triphid teeth and under *pars incisiva*; palps narrow, with distal segment very long.

Palp of maxilla II (Fig. 2 *E*) provided with 3 pennate spines on the basal segment and 5—6 long spines on the distal margin of the distal segment straightly cut.

Peraeopods all with strikingly uniform structure and size : very long and thin (Fig. 2 *F*) with trisegmented tarsus. Dactylus long, provided with a claw finer than a seta; propodite by 1/3 shorter than the carpopodite (Fig. 2 *G*). It is worth mentioning in this tarsus a very particular structure : among the specific setae, serrated on the basal portion and naked in the remaining ones, generally straight (*s*), there are attached some phaneral formations, fine, long, of equal diameters but differently curved, especially on the dactylus basis. Had they not been present in all peraeopods, and had their variability and distribution not shown a certain constancy, they might have been taken for some parasitic filaments.

Exopodites of approximately the same length as the peraeopods, presenting at the basis a small distal outer quill, only at the 3rd pair, and a transparent thin skin at the insertion site. This latter formation is present in all peraeopods and appears as largely common within the genus. Zimmer [7], the first to point out this formation, wrote in 1917, in connection with his *Tenagomysis* sp. : "An den Gangfüssen war auf den Basipoditen eine schlauchförmige kieme vorhanden". Whether it has indeed a respiratory role — that of "gills" — remains to be seen.

Pleopods, 5 unisegmented laminae in ♀♀, present particularly strong bases in ♂♂, at all 5 pairs; the dimorphic pleopod (IV) presents at the exopodite end — hardly longer than the endopodite — a bunch of 3 phanera of the last 3 joints, rather serratedly laminated, ending approximately at the same distal level (Fig. 2 *H*).

Telson, of about the same length as the supporting pleonite, distinctly trapezoid (Fig. 2 *I*); its margins are straight, with 4—5 proximal

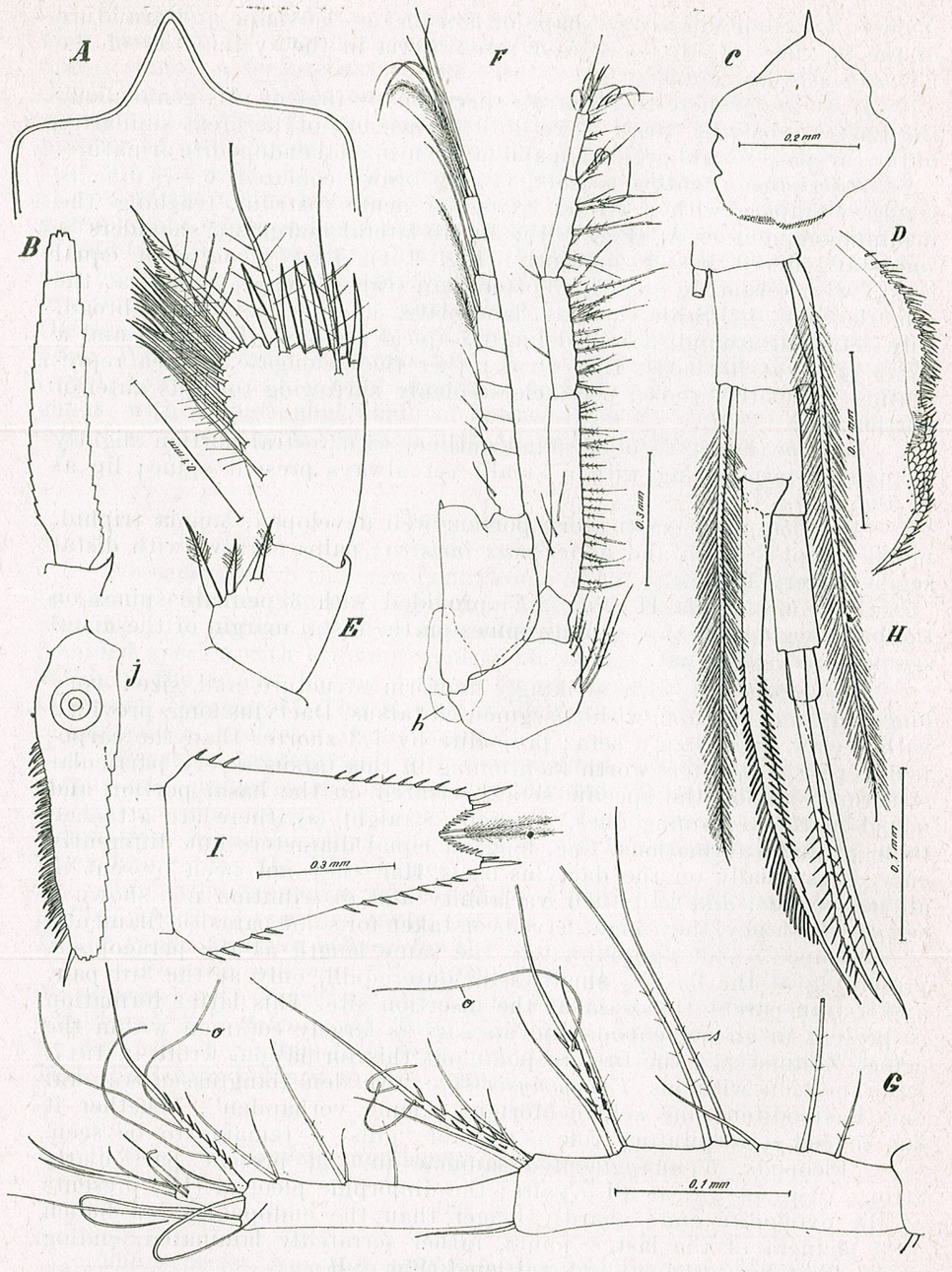

Fig. 2. — *Tenagomysis (Nouvelia) natalensis mombasae* n.sp.♂♀. *A*, rostral portion of carapace; *B*, scale of A_2; *C*, labrum with rostral spine; *D*, posterior portion of labrum; *E*, palp of maxilla II; *F*, peraeopod I; *h*, hyaline portion (respiratory) of the exopodite basis; *G*, non-segmented dactylo-, propo- and carpopodite of peraeopod I (as also II—V); *s* = particular phanera; *o*, oostegite; *H*, tip of exopodite of pleopod IV ♂; *I*, telson (♀); *J*, endopodite of uropod (♀) (orig.).

spines, followed by a hiatus exceeding half the lamina, and another 8—9 spines of the same size, in the terminal half; *lateral spines much smaller than the strong apical spines.* Sinus straight, of little depth (reaches the joining level of the antipenultimate pair of lateral spines), with about 28—30 laminae and 2 plumose setae distinctly exceeding the tips of the apical spines.

Endopodite of the uropod slightly exceeds the telson length and is provided with 50—54 thick spines, not equal; the only isolated spine at the distal portion of the lamina (almost subapically inserted) (Fig. 2 *J*). Exopodite 1/5 longer than endopodite.

Colour intensely brown, as burnt, resembling that of *Mysidopsis kenyana.* Only flagelli of antennae, peraeopods extremities with their exopodites flagelli, and the tip of the tail are transparent.

Material examined, locality: 4 specimens: 2 ♂♂, 1 ♀ ovigerous, 1 juv. Beach of Mombasa, Kenya, 0.5 m. depth, in puddles remained after ebb, 17.III.1972.

Size: ♂, ♀ = 6.8—7.2 mm.

Holotype ♂, in the coll. of the "Gr. Antipa" Museum, no. 224; allotype ♀, ditto, no. 224 b; paratypes, ditto, 1 ♂ dissected, 1 ♀, 1 juv. no. 225.

The species is cited for the first time after the original description; on this occasion the description of *T. (A.) natalensis* is completed by its variant *mombasae*, pointing out the dimorphism of pleopod IV and the structure of the maxilla 2 palp; it is also for the first time that the species ♀ is described, fulfilling, by a fortunate haphazard, the very wishes of the author: "It is to be hoped that further exploration of South African waters may yield other specimens of this interesting species so that the female may be described and the fourth pleopod of the male may be studied".

This widening of the distribution area of the species by some thousands of km. north shows that we are dealing with a species broadly spread on the west coast of the Indian Ocean. Affinities with southern New Zealand species.

Species of shallow water, benthic, homochromous brown with the brown background of the algal detritus where he lives along with *Mysidopsis kenyana* — with which it has many colour and appearance affinities —, with *Anisomysis maris rubri* Băcescu [1] and *Siriella brevicaudata* Paulson.

Remarks. Nouvelia natalensis mombasae nobis resembles — as to the telson shape and the armature of the distal margin of the maxilla II palp — *T.* (*Nouvelia*) *nigeriensis* O. Tatt. 1957; it can be readily distinguished by its only 2 dimorphic setae at the exopodite of the fourth pleopod ♂ and especially by the endopodite of the uropod provided with minute spinules and not 1/3 of the lamina width.

From the type species it differs by its shorter telson with straight margins and by the larger eyes exceeding the carapace margins by almost the whole thickness of the cornea.

The hiatus among the spines on the telson margins is not "a sign of immaturity" as stated by O. Tatt. (1957, p. 182) in connection with the 6 mm. ♂ of her *T. nigeriensis*, but a good characteristic of a species group *Nouvelia*.

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SOME RECONSIDERATIONS ON THE ZOOGEOGRAPHY OF THE EURO-MEDITERRANEAN FRESH-WATER FISH FAUNA

BY

PETRU BĂNĂRESCU

The numerous contributions published during the last 12 years on the systematics and distribution of the fresh-water fishes of Europe and adjacent areas led to a reconsideration of the author's 1960 ideas on the zoogeography of the European fish fauna. Among others, *Rutilus* and *Chondrostoma* proved not to be related to any genera from western North America; the European and West Asian *Barbus* and *Capoeta* are closer to some East Asian, not to Indian genera; the Percidae seem to have originated from Europe, the Euro-Siberian genera of Leuciscinae from Siberia. The contribution of East Asia to the building of the European fish fauna appears greater, that of West Asia smaller than formerly believed.

Europe and a few adjacent areas (North-West Africa, most of Asia Minor, Transcaucasia, North Iran, Turkmenistan, most of the Syr- and Amu-Dar'ya drainages, lake Issyk-kul) represent a natural zoogeographical unit (usually considered a subregion) within the Holarctis. Berg [10] [11] gives to this subregion the name Mediterranean; I [6] proposed that of Euro-Mediterranean. In the above-listed paper, I discussed in detail the zoogeography of the fresh-water fishes inhabiting this area, mainly the regional distribution of the species, the relations with other fish faunas and the probable origin and history of the Euro-Mediterranean fish fauna.

New contributions to the systematics and distribution of the fresh-water fishes from Europe and adjacent areas were published since 1960: papers by Almaça on the fishes from Portugal [2] and especially on the genus *Barbus* from the same country [4] and from north-western Africa

[3] [5], two contributions on the fishes from Greece, including the description of a new species and subspecies by Stephanidis [26] [27], besides Stephanidis' 1950 paper [25] not recorded in my 1960 study, a review of the Cyprinidae from Turkey [16], a revision of the noemacheiline loaches [7] and of the *Chondrostoma* species [17] from the same country, a review of the Cobitidae from West Asia [8], Turdakov's book on the fishes from Kirghizia [30] and Abdurakhmanov's [1] one on those from Azerbaijan, Karaman's revision of *Capoeta* (*Varicorhinus* partim) [14] from West Asia and of all Barbinae [15] from the Euro-Mediterranean and West Asian areas, that of the *Leuciscus* s.str. from the mid-Asian part of the Euro-Mediterranean subregion [28], the discovery of an endemic subspecies of the South-East European *Barbus cyclolepis* in the Vistula drainage [21] [22]. Quite recently, Trewavas [29] showed that the right generic name of the species till now listed as *Phoxinellus* is *Pseudophoxinus*, that of those listed as *Paraphoxinus* being *Phoxinellus*. Collette's revision of the system of Percidae [12], the several papers by Bănărescu and Nalbant (summarized in their recent monograph [9]) on the mainly East Asian Gobioninae, those by Bănărescu on the Cultrinae and Barbinae from East Asia, the still unpublished contributions by Bănărescu and Nalbant on the Noemacheilinae, especially from East and High Asia, led to a reevaluation of the concepts on the relations between the fish fauna of Europe and that of North America, respectively East Asia (and partially West Asia, too).

In the light of these new data, several of the opinions included in my 1960 paper have to be revised.

GENERAL DISTRIBUTION OF THE FISH GENERA

The following modifications are to be brought to the review of the genera presented on p. 17—19 in my above quoted paper:

Rutilus is not an Euro-Sibero-West American, but exclusively an Euro-Siberian genus; the so-called *Rutilus* species from western North America belong to genera not even related to it.

According to M. Karaman [15], the following genera of Barbinae are restricted to the limit area between the Euro-Mediterranean subregion and West Asia: *Bertinius*, *Mesopotamichthys*, *Carassobarbus*, *Typhlogarra*, *Hemigrammocapoeta*, *Tylognathoides*, *Kosswigobarbus*, *Hemigarra*; yet the first-named seems to me hardly distinguishable from *Barbus*.

The loach *Sabanejewia* deserves full generic rank; its peculiar range includes the Po drainage in North Italy, the Danube, Dniester, Don and Kuban drainages in the Black Sea basin (but not North Anatolia and apparently neither the Dnieper drainage), the drainages of the northern tributaries of the Aegean Sea (from the Vardar to the Maritsa), those of the Vistula and Neman in the Baltic Sea basin, the northern slope of the Caucasus, eastern Transcaucasia and North Iran in the Caspian Sea basin, the rivers from Turkmenistan, the Syr- and Amu-Dar'ya drainages; the main species, *S. aurata*, was recorded also from the Lower Euphrates river [8], but after a single specimen which may have been mislabelled.

The genus *Noemacheilus* s. lato proved to be a heterogeneous assemblage; its only three European species belong to *Orthrias*, a genus occurring also in Siberia, northern East Asia and West Asia; the West Asian *Oxynoemacheilus* Bănărescu & Nalbant 1967 is a synonym or a subgenus of *Orthrias*. The species *N. lendli* from North and Central Anatolia (Euro-Mediterranean subregion) seems to represent a distinct genus: *Turcinoemacheilus* and *Paracobitis* are West Asian genera, while the mainly Indian and South-East Asian *Schistura* reaches southern Turkey (*S. argyrogramma*), but not the Euro-Mediterranean subregion; species belonging to the High Asian generic groups occur in the upper and partly middle Syr- and Amu-Dar'ya drainages.

Parasilurus (with one species in western Greece, all the others in East and South Asia) is now considered a synonym of *Silurus* [13].

RELATIONS WITH OTHER FRESH-WATER FISH FAUNAS

Nothing has to be added concerning the relations of the Euro-Mediterranean and the Siberian fish fauna.

Concerning the relations with the East Asian fauna, it must be specified that all presumed East Asian "Abramidini" (such as *Toxabramis*) actually belong to the Cultrinae; the Abramidini (a tribus of the Leuciscinae) are confined to Europe, eastern North America and parts of West Asia (with fossil record from Siberia). The Chinese so-called "*Acanthobramis*" represent a distinct genus, *Pseudobrama*, belonging to the exclusively East Asian Xenocypridinae. The East Asian *Aphyocypris* is not related to the European *Leucaspius*, as formerly assumed [11] [6], but to other East Asian genera.

Four more cases of Euro-East Asian (or West-East Palaearctic) disjunctions must be added to those listed in 1960:

1) *Gobio* — subgenus *Rheogobio*: two species in Europe (*uranoscopus* in the Danube drainage, *ciscaucasicus* on the northern slope of the Caucasus), one in East Asia (*rivuloides*, Hwang-ho drainage);

2) *Gobio* — subgenus *Romanogobio*: three species in the Euro-Mediterranean subregion (*albipinnatus*, from the Danube to the Volga and Vistula, *kessleri*, in the Danube, Dniester, Vistula, Vardar, *persus*, in eastern Transcaucasia and North Iran), four in East Asia (*shansiensis*, *johntreadwelli* and *amplexilabris* in the Hwang-ho, *tenuicorpus* in the Amur and Lwang-ho drainages);

3) *Barbus* s.str. in Europe, North-West Africa and West Asia — "*Barbus*" *grahami* and perhaps other Barbine species in western China;

4) *Capoeta* (sensu M. Karaman) in Anatolia, Transcaucasia, etc. and West Asia — *Onychostoma* in East Asia.

On the contrary, the presumed disjunction of *Leucaspius* — *Aphyocypris* and *Yaoshanicus* listed in 1960 is not actual; as mentioned above, these genera are not related to one another.

West Asia appears to be closer, ichthyologically, to the Euro-Mediterranean area than I presumed in 1960. *Barbus* s.str. is widely distributed in both areas and apparently not outside them; *Capoeta* occurs in Anatolia, Transcaucasia and the Aral Sea drainage within the Euro-

Mediterranean subregion and in most of West Asia. Both genera are absent from the eastern part of West Asia (Afghanistan, Seistan, etc.) and their closest relatives seem to live in East Asia : "*Barbus*" of the *grahami* group and *Onychostoma*; this fact suggests a former occurrence of *Capoeta* in Europe and of both genera in Siberia, too. Several mainly European genera of Leuciscinae occur also in West Asia : *Rutilus*, *Pseudophoxinus* (= *Phoxinellus* auct.), *Chondrostoma*, *Aspius*, *Alburnoides*, *Chalcalburnus* and especially *Alburnus*. Other genera are mainly or exclusively West Asian, partially reaching central Anatolia : *Acanthorutilus*, *Acanthobrama*; they too belong to the Leuciscinae, a mainly Euro-Siberian subfamily.

Concerning the relations between the fish faunas of Europe and North America, it must firstly be mentioned that the Cyprinidae from the two continents are less related than I presumed in 1960. *Chondrostoma* and *Rutilus* actually are not close to the West American *Acrocheilus* and *Siphateles*, respectively *Hesperoleucus*; *Leuciscus* seems not to be close to the eastern North American *Clinostomus*; only *Abramis* is related to *Notemigonus* from eastern North America, while the only genus common to both continents is the circumpolar and rather cold-adapted *Phoxinus* (= *Chrosomus*).

Neither the secondary fresh-water Cyprinodontidae from both continents are as closely related as formerly assumed; at present, all European, Circummediterranean and West Asian genera are ascribed to a peculiar subfamily, Aphaniinae, distinct from the American Cyprinodontinae, but nevertheless closer to it than to any other subfamily.

The affinities between the faunas of both continents are better marked in the three primary fresh-water families of non-Ostariophysi : Esocidae, Umbridae (the genus *Umbra* is confined to eastern Europe and eastern North America) and Percidae. The last-named family is mainly European and eastern North American, its quite few Siberian representatives occurring also in Europe or being rather recent offshoots of European species. The Percidae include more European basic groups, but much more American species.

DISTRIBUTION OF GENERA AND SPECIES WITHIN THE EURO-MEDITERRANEAN SUBREGION

The contrast between the fish fauna of "Central Europe" (including also West and East Europe, north of the Pyrenees, Alps, Balkans, etc.) and the southern part of the subregion, which I pointed out in 1960, remains valid, as well as the fact that the Central European fauna centers in the Danube drainage and decreases both west- and eastwards. But towards the West there is only a gradual diminution of the number of species, the only fish occurring in West Europe and absent from the Danube being *Chondrostoma toxostoma* (Loire, Rhône, Garonne, but also Spain), besides some recent marine intruders. Two primary fresh-water Cyprinidae absent from the Danube drainage occur north of it : *Barbus cyclolepis* (with disjunct range : Vistula, then South Bulgaria,

Caucasus, etc.) and *Phoxinus percnurus* (a Siberian species reaching to the lower Vistula and Odra). A decrease of the number of species is noticeable also east of the Danube, but it is partially compensated by the occurrence of some species not living in the Danube : *Leuciscus danilewskii*, *Percarina demidoffi* a.o.

The southern part of the subregion is inhabited by a fish fauna rather poorer than the Central-European; many genera are absent from these southern areas, while other genera inhabit only a part of them, especially the Balkan Peninsula and North Anatolia. In the southern parts of the subregion live many fishes absent from Central Europe; some of them belong to genera occurring also in Central Europe, such as *Rutilus*, *Chondrostoma*, *Barbus* a.o. I considered [6] as an exclusively southern species-group the *Chondrostoma* — subgenus *Machaerochilus*, including the species *toxostoma*, *genei*, *phoxinus* and *cyri*. Actually *cyri* belongs to *Chondrostoma* s.str., being synonym with the sympatric *C. schmidti* [1]; but another species must be added to *Machaerochilus* : *Ch. reiseri*, which I considered [6], according to S. Karaman's 1956 opinion, as doubtful, but which I recently examined and which proved to be a "good species". Yet there is another exclusively southern group of species : the *Rutilus* — subgenus *Orthroleucos*. It includes the following forms, usually considered species : *arcasii* (Spain and Portugal), *rubilio* (Italy and West Balkan), *macedonicus* (Vardar drainage), a North-West Anatolian form ascribed by Ladiges [16] to *rubilio*, then *atropataenus* and *sojuchbulagi* (both in the Kura-Araxes drainage, East Transcaucasia). These forms are so close to each other that they can be considered subspecies of a polytypic species, except the two last-named which are specifically distinct, occurring sympatrically.

There are in the southern part of the subregion also genera not occurring in Central Europe, either endemic or living also in parts of West Asia. Some of them belong to the Leuciscinae, having thus northern affinities; others belong to the Barbinae or Cobitidae (Noemacheilinae), having rather West Asian (or East Asian) affinities.

RECORD OF FOSSIL FISHES

I summarized on p. 49—55 of my 1960 paper the data on the fossil fishes from Europe and Siberia. Remarkable contributions were published since then, especially by N. Obrhelová, among others a recent comprehensive study on *Tinca* and related tertiary genera [20]. This genus includes a single recent species in Central Europe and West Siberia; its position within the Cyprinidae is doubtful : it is usually included within the Leuciscinae, yet osteologically it seems closer to the Cyprininae [23] [24]. Obrhelová's study suggests that *Tinca* is the last survivor of a distinct phylum (subfamily) within the Cyprinidae, which has undergone a long evolution in Central Europe (and probably in Siberia, too).

THE ELEMENTS OF THE EUROPEAN FRESH-WATER FISH FAUNA

I give to the term "element" quite another understanding than De Lattin [18], who meant the origin and especially the postglacial dispersal centre of species. I meant [6] the origin of genera. The following modifications of my 1960 tentative to group the fish genera from Europe in genetic elements are necessary.

The "North American element". I included under this name the genera *Umbra*, *Esox*, *Perca*, *Lucioperca* (right name: *Stizostedion*), then *Rutilus* and *Chondrostoma*. The assumed kinship of both last-named genera with western North American genera proved wrong. The other four genera actually occur both in Europe and in North America; there is no ground for concluding in which continent they did originate. A special attention is to be granted to the family Percidae as a whole, which includes more basic groups in Europe but more species in North America; it seems that it originated in Europe, but had better survival possibilities in North America. Quite probably, the family dispersed firstly by a North Atlantic route; but the recent range of *Perca* and *Stizostedion*, as well as that of *Esox* and *Umbra* can better be explained by a dispersal through Siberia and a North Pacific land bridge.

To the "East Asian element" must be added the genera *Barbus* and *Capoeta*, whose affinities with East Asian genera were already mentioned, and the Noemacheiline genus *Orthrias*, and its offshoots: *Oxynoemacheilus* and perhaps the generic group including *N. lendli*.

Excluding *Barbus* and *Capoeta*, the "West Asian" element becomes quite poor; it includes *Garra*, may be a few other Barbinae, perhaps *Silurus* s.str. and among the Noemacheilinae *Paracobitis* and *Schistura*, both having South Asian affinities.

The autochthonous "Euro-Siberian element" includes the largest number of genera; to those mentioned in 1960 one must now add *Rutilus* and *Chondrostoma* which, as formerly mentioned, have no closer kinship to any North American genera. A special mention for the genera *Tinca*, on whose old age and long evolution in Europa I already insisted, and *Pelecus*, which seems to belong to the subfamily Cultrinae and to be related especially with a South-East Asian group of genera (*Oxygaster*, etc.). This "autochthonous" Euro-Siberian element actually has a double origin: the Cyprinidae genera (and the few genera belonging to primary marine families included within this element, such as *Eudontomyzon*) originated in Siberia and have remote (or even close) kinship to the East Asian fauna, while the Percidae (*Acerina*, *Percarina*, *Aspro*, *Romanichthys*) originated in Europe, when this continent was still connected to North America. The only suprageneric taxon originating from and endemic to Europe is the Percid tribe Romanichthyini (*Aspro* and *Romanichthys*), whose range includes the drainages of the Danube, Dniester, Vardar and Rhône.

THE PROBABLE HISTORY OF THE FISH FAUNA

The Percidae appear to be the older primary fresh-water fish family in Europe (besides perhaps some extinct families); they probably originated in this continent and soon reached North America through a still existing North Atlantic land bridge. The Esocidae and Umbridae are old families, too, but their dispersal from Europe to North America or vice versa may be recent.

But the bulk of the European fish fauna came from Siberia: the East Asian element and the many genera of Cyprinidae ascribed to the Euro-Siberian element. All East Asian genera reached Europe (and from here West Asia or parts of Middle Asia) by the northern, Siberian route; this is true also for *Barbus* and *Capoeta*, eventually their ancestors. A direct dispersal from East Asia to West- and Middle Asia can be accepted only for High Asian genera: Schizothoracini and Noemacheiline loaches (among others for the ancestor of "*Noamacheilus*" *kuschakewitschi*, which seems close to the Upper Yangtze *Oreias*).

There seems to have been an uninterrupted penetration of Siberian and East Asian genera in Europe. To the oldest Siberian invaders belong *Pseudophoxinus* (which reached North-West Africa and became extinct from Central Europe), the ancestors of *Tinca* (whose old age in Europe was already mentioned), *Rutilus*, *Chondrostoma* (both more speciose in South-West Europe) a.o.; to the youngest invaders belog *Leuciscus* s.str., the subgenus *Idus*, then *Abramis*, *Blicca* (both now extinct in Siberia), etc. To the older East Asian invaders belong the ancestors of the warm-adapted *Barbus* and *Capoeta* and of *Sabanejewia*; younger invaders are the gudgeons subgenera *Rheogobio* and *Romanogobio*, the *Cobitis* of the *elongata* group and perhaps *Cyprinus*; to the youngest wave of East Asian immigrants belong *Gobio* s.str., *Rhodeus* and the group *Cobitis taenia*.

My 1960 assumption that the South European peninsulas received independently their fish fauna from the North retains its validity.

Many facts mentioned in this paper (the probable East Asian origin of *Barbus* and *Capoeta*, the penetration of Noemacheiline loaches of the *Orthrias* group and of several Leuciscinae rather far into West Asia) suggest that the fish fauna of this area is closer to the European (e.g. Holarctic) than to the Oriental (South Asian) fauna. Also the fauna of fresh-water mussels, of operculate snails (*Theodoxus*, *Melanopsis*), of fresh-water crabs (*Potamon* s.str.) from West Asia has European affinities. My 1960 assumption that West Asia represents a subregion of the Sino-Indian region must be reevaluated.

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OCTOLASIUM (*OCTODRILUS*) *ROBUSTUM* NOUVELLE ESPÈCE DE LUMBRICIDAE ET SES AFFINITÉS

PAR

VICTOR POP

In this paper is described a new Lumbricidae species of large size, occuring in some restricted area in mountains of volcanic origin situated in the northern part of Transylvania, Romania. Its differences and similarities with other related species are described.

Sur les versants des montagnes d'origine volcanique Gutîi et Ţibleş, de la Transylvanie de nord, vit une espèce de Lumbricidae de grande taille, inconnue encore par la science. Je la nomme, à cause de sa grande taille, *Octolasium* (*Octodrilus*) *robustum*.

La description de cette espèce se base sur l'étude de 15 exemplaires adultes, dont 10 sont complets.

Morphologie externe. La longueur des exemplaires fixés en formol et contractés varie entre 200 et 300 mm. Le diamètre du corps varie entre 13 et 15 mm dans la région génitale et entre 8 et 10 mm dans la région postclitellienne. Le nombre des segments varie entre 224 et 253. Deux exemplaires adultes seulement ont 145, respectivement 175 segments. Ceux-ci sont biannelés.

L'extrémité antérieure du corps est conique, la région génitale est gonflée, le reste de la moitié antérieure du corps est cylindrique, la moitié postérieure est aplatie et élargie subterminalement.

Le corps a la couleur blanchâtre, la face dorsale, surtout dans la région antérieure, étant pigmentée en brun-grisâtre.

Le prostomium est épilobique, ouvert ou fermé.

Le premier pore dorsal est situé dans la scissure 13/14 chez les exemplaires des monts Gutîi, et dans la scissure 12/13 chez les exemplaires des monts Ţibleş. Les néphridiopores sont situés dans la partie antérieure des segments, juste au-dessus de la ligne des soies *b*. Les pores

des spermathèques, en nombre de 5 paires, sont situés sur des papilles bien visibles dans les scissures 6/7—10/11, dans la ligne des soies *c*. Les pores femelles sont situés sur le segment 14 juste au-dessus des soies *b*. Les pores mâles sont situés sur le segment 15, au moitié de l'intervalle *bc*. Tant les pores mâles, que les pores femelles sont de petites fentes à peine visibles, sans aires glandulaires.

Les soies sont relativement fines. Celles de la région postclitellienne sont plus courbées que celles de la région antérieure. Leur nodule est médian et leur extrémité distale est desquamée en forme d'écailles. Elles ont une longueur de 0,96—1,2 mm et leur diamètre au niveau du nodule

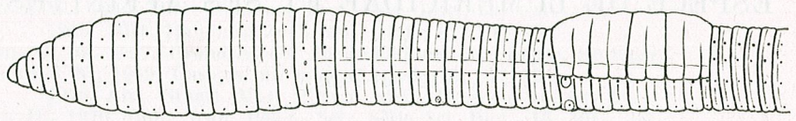

Fig. 1. — *Octolasium* (*Octodrilus*) *robustum* n.sp., région antérieure vue de profil.

est de 0,07—0,1 mm. Les soies *a* du segment 22 et les soies *a* et *b* du segment 30 sont entourées de papilles, respectivement d'aires glandulaires, mais elles ne se distinguent des autres soies que par ce qu'elles sont plus fines et plus étroites. Une soie génitale du 30^{e} segment a une longueur de 0,88 mm et un diamètre de 0,06 mm. Les soies sont écartées. Les intervalles relatifs qui séparent les soies sont les suivants: Sur le 10^{e} segment, $aa : ab : bc : cd : dd = 6 : 2{,}5 : 3 : 3 : 20$. Au milieu du corps, $aa : ab : bc : cd : dd = 6 : 3{,}5 - 4 : 1{,}8 - 2 : 1{,}4 - 2 : 11$. Donc l'intervalle dorsal (*dd*) est un peu plus petit que 1/2 de la circonférence du corps. L'intervalle ventral (*aa*) s'accroît vers l'extrémité postérieure du corps.

Pendant l'anesthésie du ver avec une solution alcoolique de 10°, dans le but de le tuer, par la contraction de la musculature de la paroi du corps, les intervalles entre les soies se modifient continuellement. En particulier les intervalles *bc* et *cd* deviennent tantôt l'un, tantôt l'autre, plus grands. Il en résulte la faible valeur qu'on peut accorder au caractère donné par les intervalles relatifs entre les soies.

Le clitellum, en forme de selle, est situé sur les segments 1/2 29—38 (=9½), rarement sur les segments 29—38 (=10). Il est très épais, mais toujours moins développé, moins épais, sur le segment 29. Sa couleur est rouge brique. Les tubercules de puberté, en forme d'étroites proéminences de même largeur et coupées par les scissures, accompagnent le clitellum sur ses deux côtés le long des segments 30—38 (=9), dorsalement par rapport à la ligne des soies *b*, mais n'atteignant pas la ligne des soies *c*. Elles sont plus ou moins nettement délimitées. Leur couleur est un peu plus claire que la couleur fondamentale du corps. Les nervures génitales sont peu prononcées.

Anatomie. Les parois du corps sont épaisses, à musculature longitudinale de type penné. Les dissépiments 5/6—14/15 sont fortement épaissis, en particulier les dissépiments 13/14 et 14/15. La glande de Morren a trois paires de gonflements latéraux peu prononcés dans les segments 10, 11 et 12, les diverticules de Perrier, du segment 10, étant

plus grands. Le jabot est compris dans les segments 15 et 16, le gésier dans les segments 17—19.

Le typhlosolis se termine dans un des segments 164—170.

Les cœurs moniliformes, en nombre de 6 paires, sont compris dans les segments 6—11.

Deux paires d'entonnoirs vibratiles dans les segments 10 et 11. Les canaux déférents ne forment pas d'épididymes. Quatre paires de vésicules séminales dans les segments 9—12. Celles des deux premières paires ont la forme de cornue (de laboratoire) au cou (la partie distale) recourbé. Celles des deux dernières paires ont la forme de coussinet avec des lobes grands et peu nombreux. Les spermathèques globuleuses, grandes et sessiles, en nombre de 5 paires, sont situées dans les segments 6, 7, 8, 10 et 11. Dans les segments 10 et 11 il y a deux paires de capsules séminales périœsophagiennes, aux parois épaisses, renfermant ensemble entonnoirs vibratiles, vésicules séminales, spermathèques et cœurs des segments 10 et 11.

Ecologie et éthologie. Cette espèce n'a été trouvée que dans les forêts de feuillus et dans les sols bruns et bruns acides, sur les versants de montagnes volcaniques, à l'altitude de 700—1 000 m.

Pendant la journée, surtout en temps sec, le ver se retire dans ses galeries souterraines, mais pendant la nuit ou en temps humide il remonte à la surface et s'abrite sous de grosses pierres, avec le corps enroulé. Il peut être trouvé ici raide de bon matin. Soulevant avec soin la pierre sous laquelle il est abrité, on peut le capturer. Mais si on marche à pas lourds dans le voisinage ou si on met plus de temps à soulever la pierre, il se retire dans ses galeries, d'où il ne peut être extrait ni même avec la pioche ou le pic. Les tentatives de le maintenir en vie dans le laboratoire, dans des récipients contenant du sol humide, n'ont pas réussi, comme dans le cas des espèces de petite taille.

Parasites. Ce ver est parasité par un Monocystidae qui vit dans la cavité des segments génitaux et dont les spores abondent dans les vésicules séminales.

Terra typica. Les monts de Gutîi et Mogoşa près de Baia Mare département de Maramureş, Roumanie, à 700—1 000 m d'altitude. Monts de Ţibleş, département de Bistriţa-Năsăud (mont de Ştegiorul à 900 m d'altitude et mont de Păltiniş, à 1 000 m d'altitude).

Date du prélèvement. Monts de Gutîi et Mogoşa : 2.VII.1955, legit T. Persecă et V. V. Pop. 7.VII.1966, 17.IV.1968, legit V. Pop. IV.1968, legit A. Micluţa. 30.III.1969, legit Vasile Pop. 6.V.1970, legit V. Pop. VI.1972, legit Vasile Pop. Monts de Ţibleş : V.1960, legit Stelian Cicio.

L'holotype est déposé au Muséum d'Histoire Naturelle « Gr. Antipa » de Bucarest. Un paratype est déposé au Muséum zoologique de Cluj, un autre dans la collection des Lumbricidae de l'Institut de Zoologie systématique de l'Université de Budapest et un troisième dans la collection des Lumbricidae de l'Institut de Morphologie animale de Moscou.

Affinités. Par la position du clitellum, des tubercules de puberté, par le nombre de paires de spermathèques et par la position de leurs pores, principaux caractères spécifiques de la famille des Lumbricidae, *Octolasium* (*Octodrilus*) *robustum* ressemble plus aux espèces *O.* (*O.*) *gradinescui* Pop et *O.* (*O.*) *transpadanum* (Rosa). Il ressemble aussi à

l'espèce *gradinescui* par la présence et la position des soies genitales. En effet, l'espèce *gradinescui* a elle aussi les soies *a* et *b* du 30e segment entourées d'aires glandulaires et parfois les soies *a* et *b* des 21e et 22e segments aussi entourées de papilles glandulaires.

O. (*O.*) *robustum* diffère cependant de *O.* (*O.*) *gradinescui* par ses dimensions plus grandes, par le nombre plus grand de segments du corps, par les intervalles relatifs entre les soies, par son écologie et son éthologie et par des caractères biochimiques.

O. (*O.*) *gradinescui*, au même état de contraction que *O.* (*O.*) *robustum*, a une longueur de 135—200 mm et un diamètre de 5—8 mm. Elle a un nombre de 160—266 segments.

O. (*O.*) *gradinescui* vit dans les plaines ou sur des collines basses, dans des sols de potagers richement fumés, où il creuse des galeries, mais ne s'abrite pas sous des pierres et peut être maintenu en vie dans le laboratoire pendant longtemps dans des récipients à sol humecté.

Ainsi que nous l'avons montré dans un travail récent [1], *O.* (*O.*) *robustum* diffère de l'*O.* (*O.*) *gradinescui* aussi par le spectre des aminoacides libres, par l'azote aminique libre total et par l'activité transaminasique du tissu musculo-cutané du corps.

Les deux espèces diffèrent aussi par leur répartition géographique. *O.* (*O.*) *robustum* vit isolé dans des montagnes écartées l'une de l'autre, Gutîi et Mogoşa d'une part et Ţibleş d'autre part. *O.* (*O.*) *gradinescui* vit par contre dans les plaines ou sur des collines basses ; elle a une répartition plus large, dans les contrées situées entre les deux montagnes ci-dessus et dans la Plaine de la Transylvanie également. Cette répartition est elle aussi discontinue.

Nous avons cru que *robustum* et *gradinescui* pourraient être considérées comme deux sous-espèces d'une même espèce, mais en tenant compte de la tendance actuelle des auteurs qui s'occupent de l'étude du genre *Octolasium* et qui ont établi récemment de nombreuses espèces nouvelles peu différentes entre elles, nous considérons également les deux formes comme étant des espèces différentes.

O. (*O.*) *robustum* diffère encore plus de *O.* (*O.*) *transpadanum* tant par ses dimensions plus grandes, par le nombre plus grand des segments du corps, par la position de clitellum et des tubercules de puberté et par les intervalles entre les soies, que par son biochimisme, de sorte que nous ne considérons pas nécessaire d'énumérer ici les autres différences qui séparent ces deux espèces.

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DÉNOMINATIONS NOUVELLES POUR QUELQUES ESPÈCES DE *LITHOBIOMORPHA* (*CHILOPODA*)

PAR

Z. MATIC

Eupolybothrus graecus Matic 1970 *nomen praeocupatum*, would be *Eupolybothrus epirensis* nom. nov.; *Lithobius alpinus* Mat. et & Dărăb. *nomen praeocupatum*, would be *Lithobius alpicosensis* nom. nov.; *Lithobius bicolor* Tak. nomen praeocupatum, would be *Lithobius ansyensis* nomen novum.
The subspecies *Lithobius muticus triodontus* Mat. & Ceuca, is synonymous with *Lithobius muticus sexdentatus* Verh

En 1970 et 1971, nous avons eu l'occasion de décrire deux espèces nouvelles pour la science : *Eupolybothrus graecus* Matic 1970 et *Lithobius alpinus* Matic & Dărăbanţu 1971, sans nous apercevoir que ces noms existaient déjà.

Nous profitons de cette note pour corriger les noms de ces espèces.

1. **Eupolybothrus graecus** Matic 1970

(Fragm. Entom. 7, p. 16, 1970) — *nomen praeocupatum* — *Lithobius* (*Polybothrus*) *fasciatus graecus* Verhoeff 1899 (Verh. Zool. Bot. Ges. Wien 49, p. 454, 1899), se nommera désormais :

Eupolybothrus epirensis nom.nov.

Derivatio nominis : nous désignons cette espèce d'après la localité — Epiro (Grèce).

2. **Lithobius alpinus** Matic & Dărăbanţu 1971

(Mem. Mus. Civ. st, nat. Verona 18, p. 414, 1971) — *nomen praeocupatum* — *Lithobius alpinus* L. Koch 1862 (Die Myr. Gatt. Lithobius, p. 66, 1862), se nommera désormais :

Lithobius alpicosiensis nom. nov.

Derivatio nominis : nous désignons cette espèce d'après la localité — Alpi-Cozie (Italie).

En 1939 Takakuwa a décrit l'espèce *Lithobius bicolor*, mais son nom est aussi — un *nomen praeocupatum* — *Lithobius bicolor* Tömösváry 1879.

Nous profitons de cette occasion pour corriger aussi la dénomination de cette intéressante espèce.

3. **Lithobius bicolor** Takakuwa 1939

(Sapporo Nat. Hist. Soc. Sapporo 16, p. 32, 1939) — *nomen praeocupatum* — *Lithobius bicolor* Tömösváry 1879 (Zool. Anz. 71, p. 118, 1879), se nommera désormais :

Lithobius ansyensis nom. nov.

Derivatio nominis : nous désignons cette espèce d'après la localité — Ansyu (Japon).

Dans un travail antérieur, Matic & Ceuca (1969), nous avons décrit une forme nouvelle de *Lithobius* — *Lithobius muticus triodontus*. Après des études ultérieures d'autres matériaux, nous avons conclu qu'il s'agit de *Lithobius muticus sexdentatus* Verhoeff 1937.

En conséquence

L. muticus sexdentatus Verhoeff 1937

(Arch. Naturg. N. F. 6,2, p. 231, 1937).
syn : *L. muticus triodontus* Matic & Ceuca *n. syn.*

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Université « Babeş-Bolyai »
Chaire de Sciences Naturelles et Agricoles
Cluj, Str. Clinicilor 5—7

QUANTITATIVE CYTO-ARCHITECTONIC STUDY OF THE OLFACTORY BULB AND THE ANTERIOR OLFACTORY NUCLEUS IN *TRITURUS CRISTATUS* L.

BY

LILIANA BABEŞ and ELIZA LEONTE

Cells architectonics of bulbus olfactorius and nucleus olfactorius anterior in *Triturus cristatus* L. was investigated from the quantitative point of view. It was established that there are statistically significant differences between the means of the nuclei diameters of the neurones belonging to bulbus olfactorius and nucleus olfactorius anterior as well as to the adjacent regions.

The morphologic study of the Urodela encephalon called the research-workers' attention from at least two standpoints : the phylogenetic and the physiologic aspect. As to the first aspect, it is not yet demonstrated whether the encephalon with a primitive structure in Urodela is a primary, unevolved one, or is in fact the result of a secondary simplification following a phylogenetic regression. Herrick, who studied most thoroughly the encephalon morphology in Urodela (*Ambystoma*, *Necturus*, *Salamandra*) is more inclined to the second assumption, considering for instance that the encephalon of *Necturus* may be merely "the persistence of a larval stage resulting from a secondary return to a generalized ancestral form".

From the physiological point of view, the accurate investigation of the nervous nucleus topography and of the nervous paths may be useful for explaining certain reflex responses at this developmental step. Accordingly, the stereotaxical knowledge of the encephalon structure in Urodela is also needed, though these animals are less used in physiological experiments than the Anura.

The comparative morphologic study of the nuclei and the nervous paths may be useful in both problems. As in the genus *Triturus*, which

has a very wide distribution among the European Urodela, the encephalon was little [3] [12] and incompletely studied, we assumed that it would be useful to supplement the data known so far with new evidence, and we started therefore a study from which we are reporting in the present paper only the results obtained for the olfactory bulb and the anterior olfactory nucleus.

The topographic study of the pallium and subpallium areas of the Urodela encephalon, as well as that of the Anura and of the reptiles, rises the problem of the choice of criteria used for the delimitation of these areas, which at a first sight appear as a preventricular undifferentiated cell mass. The accurate specification of the various pallium and subpallium nervous regions is very difficult in Urodela, where some of the common criteria used in Anura, reptiles, etc. cannot be used. In full agreement with Roland Platel, who studied the reptile *Scincus*, we assume that among the criteria commonly used for the delimitation of the nervous areas and nuclei — viz. (a) the size of the neurone nuclei composing these areas ; (b) the chromaticity of these neurone nuclei ; (c) the neurone density ; (d) their arrangement in layers or small agglomerations ; (e) the presence of cell-free regions ; (f) the ventricle outline and the location of the ventricular furrows ; (g) the grey periventricular substance thickness ; (h) the course of the fibre bundles — the first five are the most significant. Among these, the most accurate and illustrative is, in our opinion, the first criterium. Our quantitative study was based upon it, and we found that by its use the nervous regions can be differentiated rather accurately within the cell mass surrounding the ventricle. We agree with the author's statement that the last three criteria are inconsistent. In the specimens tested in our study the shape of the ventricles were also very variable, and the fibre courses are dependent on the starting and reaching areas and consequently they are not a safe criterium. Moreover, for the anterior part of the newt encephalon, which is studied in this paper, the criterium of the neurone-free regions and that of the layer and small cluster arrangements were worthless. The description which follows is therefore based mainly upon the neurones size, their chromatic intensity and the neurone density in the respective zones.

As to the terminology used below, we followed that of Herrick, whose outstanding works on *Ambystoma*, *Necturus* and *Salamandra* were used for reference and comparison. Apart from some small differences, our statistical computations corroborate certain divisions into zones pointed out by Herrick in his studies. For certain homologies we made also use of H. Kuhlenbeck's works, who studied the forebrain in Urodela and Gymnophiona, of the recent studies of P. Clairembault in Anura, and those of R. Platel in the reptilian encephalon, which comprise also a quantitative study.

MATERIAL AND METHOD

Brains of *T. cristatus* removed from the cranium were used, as well as complete heads which were subjected after fixation to a treatment with 5% trichloroacetic acid solution for decalcification. The heads were fixed in Bouin-Hollande fluid, and the brains in Carnoy, Regaud

and Bouin. Paraffin inclusions were made following the conventional technique ; the pieces were cut up to 10μ thick and stained with hemalaun-erythrosine and gallocyanine. On a number of brains the Feulgen reaction was also performed, for a better distinction of the various nucleus chromaticity.

The nucleus size was directly measured with an ocular micrometer. Since most of the nuclei had an ellipsoid cross section, in the statistical calculations the mean diameter value was considered according to the formula :

$$Dm = \frac{D + d}{2}$$

where : Dm = the mean diameter
D = the large diameter
d = the small diameter.

The diameter sizes of the nuclei were expressed in μ. For every delimited zone 100 measurements were made, at regular intervals, starting from the cranial toward the caudal part of the zone. The nuclei within the studied zones were measured and also those of the close-situated zones, in order to evaluate the difference existing between them. The significance of the difference between the means of the diameters of the neurone nuclei was calculated by using the test "U", according to the formula :

$$U = \frac{M_1 - M_2}{\sqrt{\frac{\delta_1^2}{n_1} + \frac{\delta_2^2}{n_2}}}$$

where : M_1, M_2 = the means of the nuclei diameters
δ_1, δ_2 = the standard deviation
n_1, n_2 = the number of the measurements.

We assumed that the difference between the means of the nuclei diameters in the studied telencephalon areas is statistically significant when $U > 1.96$, and the transgression probability $(\alpha) < 5\%$.

$$\alpha = 1 - 2\,\Phi_0\,(u)$$

$$\Phi_0\,(u) = \frac{1}{\sqrt{2\pi}} \int_0^u e^{-\frac{x^2}{2}}\,dx.$$

RESULTS AND DISCUSSION

The olfactory bulb in *Triturus cristatus* is closely attached to the brain hemispheres, being located latero-dorsally in their rostral portion. A distinction between the bulb and the hemispheres cannot be made from the outside, and in this point the newt is more similar to *Ambystoma* than to *Necturus*, in which Herrick describes a separating furrow (fissura circularis). Each bulb is supplied with an olfactory nerve comprising 4

or 5 nervous fibre bundles. Along this nerve proceeding from the olfactory organ comes also the vomero-nasal or terminal nerve proceeding from the Jacobson's organ. The latter is a thin nerve ending in the accessory olfactory bulb, which is a formation lying in the caudal part of the proper olfactory bulb and having generally the same structure.

In the inner structure of the olfactory bulb we found the same layers as those described in Urodela by Herrick and Kuhlenbeck : the nervous fibre layer composed of the bipolar neurone axons of the olfactory mucous membrane (Plate I, Fig. 1, *1*), the glomerular layer represented by the synapses occurring between these axons and the mitral cell dendrites (Plate I, Fig. 1, *2*) (in this layer one can see among the glomeruli the peri- and subglomerular cells described by P. Ramon y Cajal and Rubaskin), and then a layer free of cells, named the plexiform layer (Plate I, Fig. 1, *3*) (molecular layer according to Kuhlenbeck). In this newt's latter layer one can see a range of cell elements which are in our opinion possibly mitral cells which moved out from their proper layer; this assumption is supported by the fact that their dimensions are similar to those of the next layer — the mitral cell layer (Plate I, Fig. 1, *4*). The last layer is built up of granular cells (Plate I, Fig. 1, *5*) which ranges up to the neighbourhood of the ventricular epithelium. All the five layers described above are named "formatio bulbaris" by Bindewald and Herrick. Kuhlenbeck includes under this name only the nerve fibre and the glomerular layers, and he calls "lobus olfactorius" the whole structure, incl. the anterior olfactory nucleus. We consider Herrick's terminology to be more fit and we maintain the name of bulbus olfactorius for the five layers. These layers are easily distinguished in the olfactory bulb even when stained by hemalaun-erythrosine. More difficult is to make a distinction between the granular and the mitral cell layers, since no limiting zone occurs between them. Nevertheless, these two zones may be distinguished rather accurately by the Feulgen reaction. The

PLATE I.

Fig. 1. — Cross section in the olfactory bulb in *Triturus cristatus* L. : *1*, nervous fibre layer; *2*, glomerular layer; *3*, plexiform layer; *4*, mitral cell layer; *5*, granular cell layer.

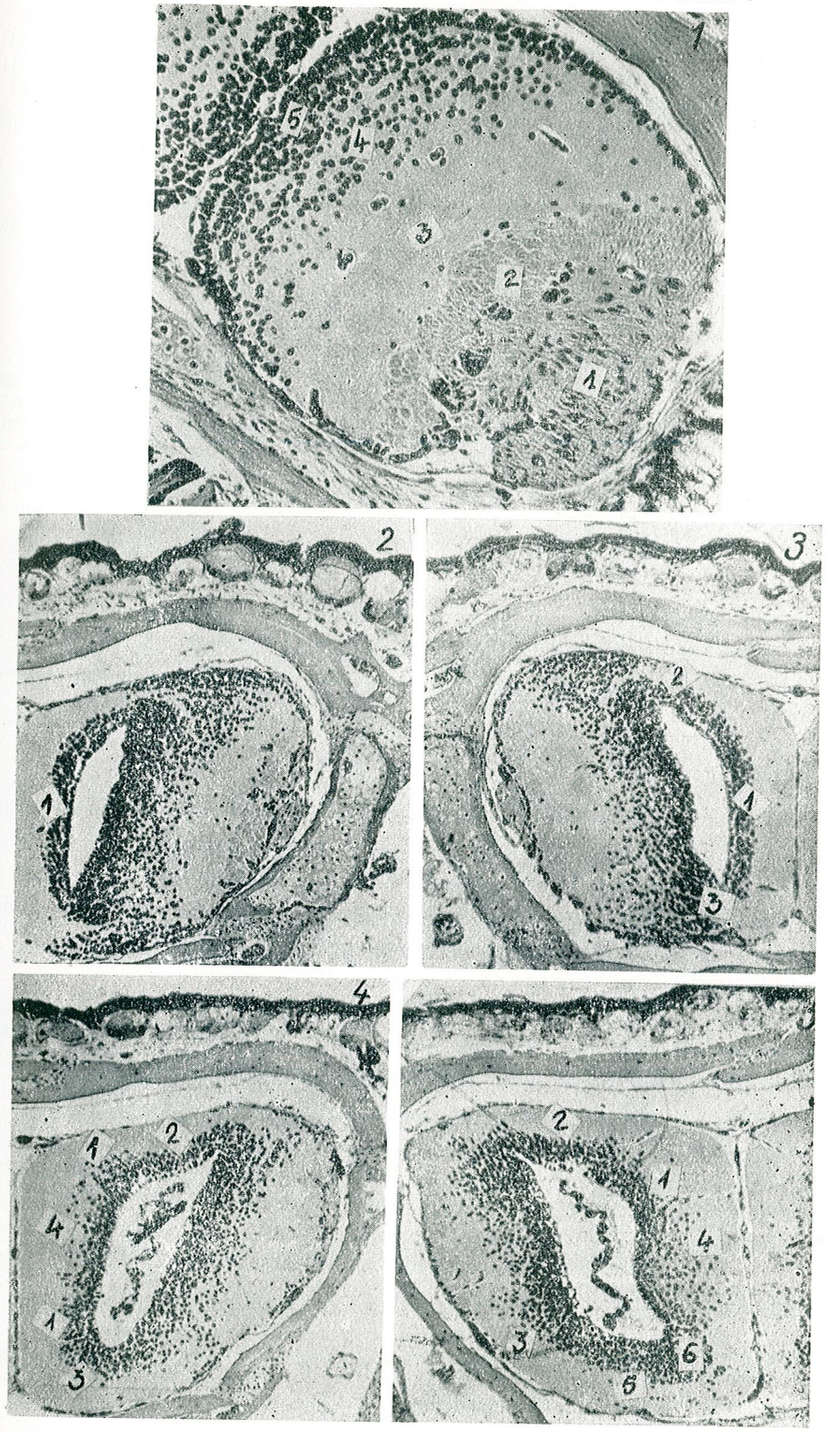
Fig. 2. — Cross section in telencephalon of *Triturus cristatus* close behind the rostral end of lateral ventricle : *1*, anterior olfactory nucleus pars medialis (a.o.n.p.m.).

Fig. 3. — Cross section in the telencephalon through the anterior third of the lateral ventricle : *1*, a.o.n.p.m.; *2*, anterior olfactory nucleus pars dorsalis (a.o.n.p.d.); *3*, anterior olfactory nucleus pars ventralis (a.o.n.p.v.).

Fig. 4. — Cross section in telencephalon at the half caudal level of the bulb formation : *1*, a.o.n.p.m.; *2*, dorsal pallium primordium (d.p.p.); *3*, a.o.n.p.v.; *4*, hippocampus pallium primordium (h.p.p.).

Fig. 5. — Cross section in the telencephalon through the posterior area of the bulb formation : *1*, a.o.n.p.m.; *2*, d.p.p.; *3*, caudal nucleus primordium (c.n.p.); *4*, h.p.p.; *5*, nucleus accumbens septi (n.a.s.); *6*, nucleus lateralis septi (n.l.s.).

PLATE I



mitral cell nuclei are larger and less chromatic, while those of the granular cells are smaller and deeper stained. The mean value of the diameters of the mitral cell nuclei is equal to 11.60 $\pm$ 0.056 μ and that of the granular cell nuclei to 9.12 $\pm$ 0.045 μ (Table 1, Fig. 6). The difference between the diameters of the two cell type nuclei is statistically significant (Table 2).

This intact stratified structure of the bulb formation is seen to extend caudally up to the half of the lateral hemisphere wall, but its

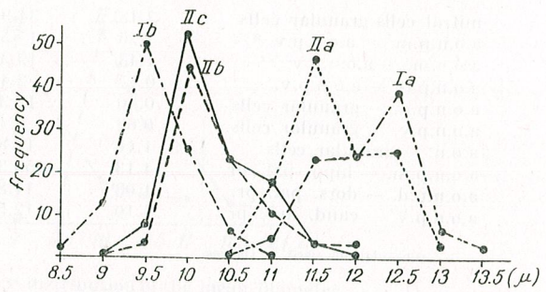


Fig. 6. — Frequency distribution of the mean diameter values of cell nuclei of the olfactory bulb : *Ia*, mitral cells ; *Ib*, granular cells ; *IIa*, a.o.n.p.m. ; *IIb*, a.o.n.p.d. ; *IIc*, a.o.n.p.v.

Table 1

Mean values of the nucleus diameters of the olfactory bulb and anterior olfactory nucleus cells in *T. cristatus* **L. (the diameter sizes are expressed in μ)**

Telencephalon zone		Number of measurements	Variation field of the diameter values (μ)	Standard deviation	Mean $\pm$ mean error (μ)
Olfactory bulb	mitral cell	100	10—13	0.56	11.60 $\pm$ 0.056
	granular cells	100	8—10.5	0.45	9.12 $\pm$ 0.045
Anterior olfactory nucleus	pars dorsalis	100	10—12.5	0.54	11.18 $\pm$ 0.054
	pars medialis	100	9.5—11.5	0.51	9.75 $\pm$ 0.051
	pars ventralis	100	8.5—11.5	0.48	9.92 $\pm$ 0.048
Total A.O.N.		300	8.5—12.5	0.84	10.28 $\pm$ 0.048
Pallium	hipp. pall. pr.	100	12.5—16.0	0.65	13.88 $\pm$ 0.065
	dorsal pall. pr.	100	7.5—10.5	0.66	8.89 $\pm$ 0.066
Caudal nucleus		100	9.0—12.5	0.75	11.02 $\pm$ 0.075

Table 2

The significance of the difference between the mean values of the nucleus diameters of the olfactory bulb and the anterior olfactory nucleus in *T. cristatus* L.

Telencephalon zones	Difference between the mean values of the nucleus diameters (μ)	"U"	Transgression probability (α)
mitral cells-granular cells	2.48	34.92	>5% (significant dif.)
a.o.n.p.m. — a.o.n.p.v. *	1.26	17.50	>5% (,, ,,)
a.o.n.m. — a.o.n.p.v.	1.43	19.32	>5% (,, ,,)
a.o.n.p.d. — a.o.n.p.v.	0.17	2.42	>5% (,, ,,)
a.o.n.p.d. — granular cells	0.80	12.30	>5% (,, ,,)
a.o.n.p.v. — granular cells	0.63	9.40	>5% (,, ,,)
a.o.n. — granular cells	1.16	17.84	>5% (,, ,,)
a.o.n.p.m. — hipp. pal. pr.	4.13	50.36	>5%) ,, ,,)
a.o.n.p.d. — dors. pal. pr.	1.03	11.83	>5% (,, ,,)
a.o.n.p.v. — caud. nuc. pr.	1.10	12.50	>5% (,, ,,)

abbreviations used in figures.

volume is progressively diminishing (Plate I, Fig. 2, *3*, *4*, *5*). Following the appearance of the lateral ventricles, the middle portion of both hemispheres is filled with the anterior olfactory nucleus cells, and the lateral portion with the formatio bulbaris (Plate I, Fig. 2). The definition of the anterior olfactory nucleus, which is a secondary olfactory centre, and the homology of its various parts in the vertebrate series are still very disputable in literature [15]. In Urodela, and particularly in *Necturus*, Herrick describes it as "an undifferentiated tissue surrounding the formatio bulbaris" and having four sections : the ventral, middle, dorsal and lateral sections, the latter two being composed of two subdivisions each : the ventro-lateral and dorso-lateral zones. In *Ambystoma*, the same author describes this formation as a "grey unspecialized substance" with three zones : the dorsal, middle and ventral zones. Kuhlenbeck, describing in general the Urodela (*Salamander*, *Triton* and *Siredon*) assumes that the anterior olfactory nucleus is a single cell mass, without any subdivision.

In *Triton*, the anterior olfactory nucleus is located on the median face of the lateral ventricle but extends also up to its dorsal and ventral ends. We observed two variation patterns concerning the disposition of the anterior olfactory nucleus as against the rostral ventricle end : a) in the first pattern, the lateral ventricle occurs in the middle of the granular layer ; under such conditions, the anterior olfactory nucleus is built up a little behind the rostral extremity of the ventricle, which is entirely surrounded by the granular cells ; b) in the second pattern, as the rostral ventricle end occurs in the median border of the granular layer, the anterior olfactory nucleus is located in the most rostral part, on the middle wall of the ventricular slit. In both cases the middle nucleus portion is the first to appear (Plate I, Fig. 2, *1*). The dorsal and ventral parts appear subsequently in the ventricle ends (Plate I, Fig. 3, *1*, *2*, *3*). All these components of the anterior olfactory nucleus may be differ-

entiated not only by their location as against the ventricle, but also by the size of their neurone nuclei. The mean diameters of these nuclei in the dorsal part of the anterior olfactory nucleus amount to 11.18 $\pm$ 0.054 μ, those of the middle portion to 9.75 $\pm$ 0.051 μ, and those of the ventral portion to 9.92 $\pm$ 0.048 μ (Table 1, Fig. 6). The difference between the neurone diameter means in these three areas is statistically significant (Table 2). The anterior olfactory nucleus gets into direct touch in its anterior part with the granular cells of the bulb formation. From

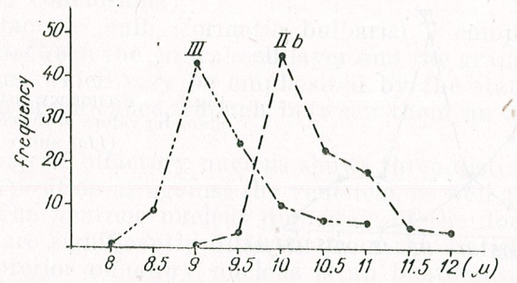


Fig. 7. — Frequency distribution of the mean diameter values of the cell nuclei of a.o.n.p.d (*IIb*) and d.p.p. (*III*).

the quantitative analysis of the nucleus diameter values we may conclude that there is a statistically significant difference between the neurones of the anterior olfactory nucleus as a whole and the granular cells, as well as between these latter and the dorsal and ventral areas of the nucleus with which they get into touch (Table 2).

The pars dorsalis of the anterior olfactory nucleus proceeds caudally, without very well definite limits, into the dorsal pallium primordium (Plate I, Fig. 4, *2*). There are however statistically significant differences between the sizes of the neurone nuclei of the two adjacent formations. The mean of the neurone nucleus diameters in the dorsal pallium primordium is equ al to 8.89 $\pm$ 0.061 μ, and that in the dorsal area of the anterior olfactory nuclei is 1.18 $\pm$ 0.054 μ (Fig. 7, Table 1). The difference of 1.03 μ between the two means is statistically significant (Table 2).

The ventral part of the anterior olfactory nucleus continues with a layer formation named by Herrick the primordium of the caudal nucleus (Plate I, Fig. 5, *3*). This formation may be distinctly seen in our sections, too, and is from the statistical standpoint significantly different with respect to the neurone nucleus sizes as against those of the ventral portion of the anterior olfactory nucleus. The difference between the mean values of the neurone nucleus diameters in these areas amounts to 1.10 μ, with $u = 12.50$ and $\alpha < 5\%$ (Table 2, Fig. 8). The middle and most caudally extended part is continued in the hippocampus pallium primordium (Plate I, Fig. 4, *4* ; Plate I, Fig. 5, *4*). The difference between the means of the neurone nuclei in these adjacent areas is conspicuous also in our sections, since the nuclei of the neurones in the hippocampus pallium primordium are remarkable for their large size (13.88 $\pm$ 0.065 μ). The quantitative study shows that this difference — 4.13 μ — is statistically sig-

nificant (Fig. 9 ; Tables 1, 2). The hippocampus pallium primordium appears in the middle area of the anterior olfactory nucleus, dividing it into two parts : the ventral and dorsal areas. The ventral part, which is located in the neighbourhood of the septum, is soon completely replaced by the hippocampus pallium primordium. The dorsal portion is longer and persists beside the dorsal part of the anterior olfactory nucleus, accompanying it along a certain distance toward the caudal hemisphere end.

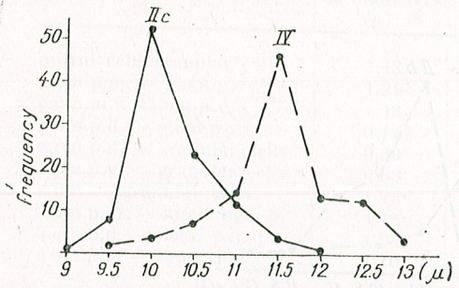


Fig. 8. — Frequency distribution of the mean diameter values of the cell nuclei of a.o.n.p.v. (*IIc*) and c.n.p. (*IV*).

The lateral field of the anterior olfactory nucleus, which is described by Herrick in *Necturus* as a cell area intermediating between the formatio bulbaris and the primordium of the pear-like pallium on the dorsal side and the striated body on the ventral side, is in our opinion

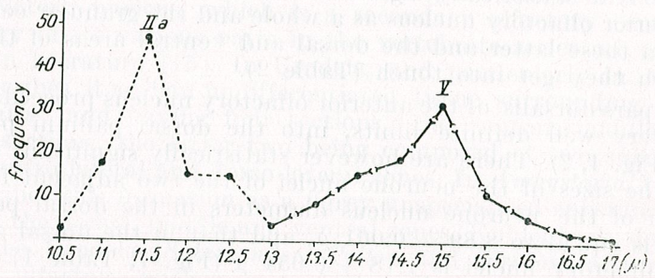


Fig. 9. — Frequency distribution of the mean diameter values of the cell nuclei of a.o.n.p.m. (*IIa*) and h.p.p. (*V*).

homologous to the Kuhlenbeck's lateral postolfactory nucleus. This zone seems to be absent in newts, the passage towards the above-mentioned formations being direct. From this standpoint, the *Triton* is more similar to *Ambystoma* than to *Necturus*, though Herrick describes in *Ambystoma* too a dorso-lateral olfactory nucleus, which is however synonymous with the pear-like pallium primordium.

The quantitative study of these areas allowed, if not an accurate definition of their limits, which is practically impossible, a higher precision in differentiating them. We could distinguish thus, in areas which were considered to be uniform masses of undifferentiated cells, such as the anterior olfactory nucleus, where several zones were stated by less accurate criteria (their position as against the shape of the ventricle),

areas clearly differentiated by the neurone nucleus sizes, which implies to a certain extent also a difference of function.

CONCLUSIONS

The quantitative cyto-architectonic study of the olfactory bulb and of the anterior olfactory nucleus in the species *T. cristatus* L. led us to the following conclusions :

1. The olfactory bulb (formatio bulbaris) is composed of the five known layers. Between the mitral cell layer and the granular cells several differences occur, which may be emphasized by the statistic calculation of the variation significance, though between them an area free of cells does not occur.

2. The anterior olfactory nucleus shows three distinct regions, with respect to their position as against the ventricle, as well as to the neurone nucleus sizes. The neurone nucleus diameters of the dorsal, middle and ventral regions are significantly different from the statistical standpoint.

3. The anterior olfactory nucleus is an anatomically distinct formation, which may be differentiated from the adjacent zones by the size of the neurone nuclei.

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RELATIONSHIP BETWEEN GLUCOSE, FATTY ACID AND KETONE BODY METABOLISM IN ISOLATED PERFUSED FROG HEART

BY

C. DOBRESCU, I. V. DEACIUC and E. A. PORA

The reciprocal effects of glucose and oleate and of glucose and acetoacetate (AcAc) on their uptake were studied in the isolated perfused hearts from *Rana temporaria*. The formation of lactate and of 3-hydroxybutyrate (when AcAc was added in the perfusion medium) was also measured. Oleate (0.5 mM) stimulated the glucose uptake and lactate formation, while AcAc (10 mM) suppressed glucose uptake with concomitant stimulation of lactate formation. The uptake of oleate and, in a lesser extent, that of AcAc was depressed by glucose (at 5.0 and 2.5 mM, respectively). The results are discussed in the light of available data on interrelation between carbohydrate and fatty acid metabolism of heart.

The idea to perform the experiments reported in this paper originated from the following experimentally established facts. On the one hand, Preiss [14] reported that in some amphibian livers there is a lack of acetoacetate (AcAc) formation. On the other hand, it has repeatedly been shown that heart muscle of higher animals, especially of mammals, uses preferentially fatty acids and ketone bodies for its energetic needs [5], [16], [19], [24]. Moreover, these substrates, when supplied in increased but still physiological concentrations, are able to suppress the glucose utilization by the heart [16], [20], [24]. Thus the heart muscle has become classically recognized as one of the main ketone body and fatty acid consumers in higher animals.

If the physiological significance of ketogenesis is taken into consideration, namely to "digest" fatty acids by converting them to products, i.e. ketone bodies, which are more easily transportable and oxidizable for several tissues, as Krebs [10] pointed out, then the question arises of to what extent heart muscle of animals lacking ketogenesis exhibits a specific metabolic pattern.

REV. ROUM. BIOL.–ZOOLOGIE, TOME 18, No 4, p. 281–288, BUCAREST, 1973

It is the purpose of this paper to study the ability of frog heart muscle to utilize fatty acids, ketone bodies and glucose for its energetic demands.

MATERIALS AND METHODS

Chemicals. Glucoseoxydase was kindly supplied to one of us (I. V. D.) by Professor M. F. Gulyi from the Institute of Biochemistry, Kiev, U.S.S.R.; peroxydase (type I, from horseradish) was purchased from the Sigma Chemical Co., St. Louis, Mo., U.S.A.; NAD and 3-hydroxybutyrate dehydrogenase, from Boehringer Mannheim GmbH, Federal Republic of Germany; bovine serum albumin (BSA) (Cohn, fraction V), from Serva Heidelberg, Federal Republic of Germany; AcAc was prepared from freshly distilled ethyl acetoacetate and standardized manometrically according to Krebs and Eggleston [8]. All other chemicals were of the highest purity commercially available.

Animals. All experiments were performed on frogs *Rana temporaria*. Other details concerning the animals are given in the legend to the figures and tables.

Perfusion of hearts. The technique of perfusion was described in detail in a previous paper from this laboratory [4]. The perfusion medium was the saline of Schüller [13] buffered with sodium phosphate (5.2 mM, pH 7.4). When glucose was present in the perfusion medium, insulin was added in a final concentration of 10 mU/ml. Before its addition to the perfusion medium, oleic acid was complexed to BSA according to van Harken *et al.* [6], to give a solution of 25 mM in oleic acid (pH 7.4) with the molar ratio of oleate/BSA of 17.4; from this solution, freshly prepared for each set of experiments, appropriate volumes were added to the perfusion medium (final volume 10 ml.) to give the desired final concentration of oleate. Simultaneously, BSA was added to the medium in a final concentration of 1 g./100 ml. In experiments where glucose consumption without oleate was to be compared to that with oleate (Fig. 2), BSA was added in the same concentration as above. In all experiments perfusion medium contained penicillin and streptomycin in a concentration of 5 and 10 mg./100 ml., respectively.

Assay of metabolites. All metabolites were assayed on appropriate aliquots of perfusion medium withdrawn at the intervals indicated in the figures and tables. Protein precipitation was done only when BSA was a component of the perfusion medium; thus, for glucose assay proteins were precipitated with perchloric acid (3% final concentration) and for lactate assay with trichloroacetic acid (5% final concentration).

Glucose was determined according to Krebs *et al.* [9] except that at the end of incubation of sample with the glucoseoxydase reagent, an equal volume of 50% (v/v) of H_2SO_4 was added under cooling [18]. Lactate was assayed by the method of Barker and Summerson [1], AcAc by the method of Walker [22], 3-hydroxybutyrate by that of Williamson and Mellanby [23] and oleic acid according to Novák [12]. The initial concentration of metabolites added to the perfusion medium was always measured. Since the duration of perfusion was long enough (8 hours) to allow the supposition of an attack by microorganisms of the various components of the perfusion medium (even in the presence of added antibiotics), for each set of perfusions an aliquot of perfusion medium was maintained in the same environment as perfusion device was, and, at the end of the perfusion period, it was analysed for metabolites initially added in it. No differences between the initial and the final concentrations of metabolites were observed.

Optical measurements were done with a Zeiss VSU-1 spectrophotometer for AcAc, 3-hydroxybutyrate and fatty acids, and with an electrophotocolorimeter (FEK-M) for glucose and lactate.

The statistical treatment of the data was done according to Student's t test. Differences at P values of 0.05 or less are considered to be statistically significant.

RESULTS

Free fatty acid concentration in blood plasma of frogs. It was important for the purpose of this work to choose an adequate concentration of oleate in the perfusion medium, since it has been reported that high

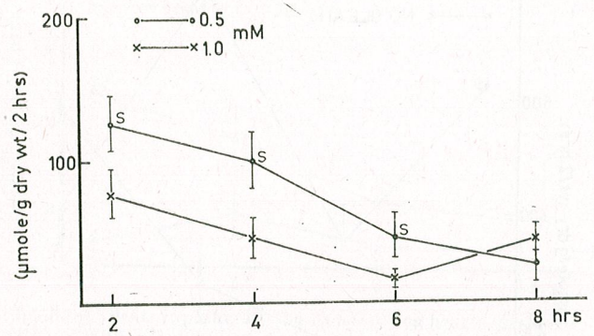

Fig. 1. — Oleate uptake by the isolated frog hearts, perfused with 0.5 or 1.0 mM oleate complexed to bovine serum albumin. Each point represents the mean value from 8 to 12 hearts with standard error (vertical bars). S denotes a statistically significant difference ($P<0.05$) between the corresponding points on the two curves. Animals of both sexes, weighing between 40 and 70 g., were caught in March, maintained in tanks with running tap water at 13°, with no food, and used for experiments in June. The perfusion was performed at 28°.

concentrations of free fatty acids can exhibit an uncoupling effect [3] [11] [15] [21]. Therefore, we determined the concentration of free fatty acids in blood plasma of fasted and of non-fasted freshly caught frogs.

Table 1

Free fatty acid (FFA) concentration in blood plasma of fasted and non-fasted frogs

State of animal	FFA in blood plasma (mM)
Fasted [1]	0.115 ± 0.002 (4)
Non-fasted [2]	0.410 ± 0.057 * (7)

The values in the table are mean ± standard error of the mean with the number of animals in parentheses. *P for non-fasted *vs.* fasted is < 0.001. [1]Animals from the same group as in Fig. 1. [2]These were caught in May and immediately (in the same day) used for assay of FFA.

On the basis of the results recorded in table 1 we have chosen for perfusion an oleate concentration of 0.5 mM. Indeed, at higher oleate concentration (1.0 mM) (Fig. 1) the rate of oleate uptake was markedly decreased.

Reciprocal effects of oleate and glucose on their uptake. As shown in Fig. 2, the rate of glucose uptake for at least 6 hours of perfusion was greater in the presence of oleate than in its absence. It is also to be pointed

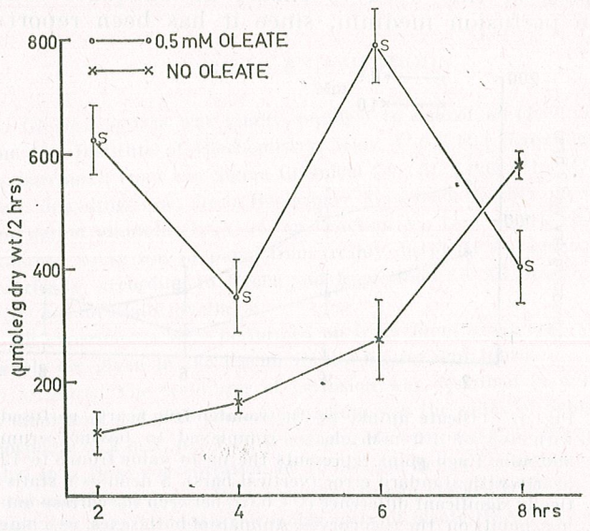

Fig. 2. — Glucose uptake by the isolated frog hearts, perfused with or without oleate and with 5 mM glucose. Each point represents the mean value from 4 to 7 hearts with standard error (vertical bars). S, as for Fig. 1. The history of animals was as described in the legend to the Fig. 1, except that the experiments were performed in June and July. The perfusion was performed at 28°.

Table 2

Lactate production by the isolated frog hearts, perfused with glucose and with glucose plus oleate

Perfusion	Lactate production (µmole/g. dry weight/8 hours)
Glucose (5 mM)	762±164 (10)
Glucose (5 mM) + Oleate (0.5 mM)	2247±234 * (5)

The values in the table are mean±standard error of the mean with the number of animals in parentheses.* The difference between the two groups is statistically significant at $P < 0.001$. Experimental conditions as described in the legend to the Fig. 2.

out that in the presence of oleate glucose uptake exhibited a clear oscillatory behaviour. Such a behaviour has also been observed in other series of experiments on isolated perfused frog heart, performed in our laboratory (unpublished data). At 8 hours of perfusion period glucose uptake was decreased in the presence of oleate. It is also seen from the data in table 2 that stimulation by oleate of glucose uptake was associated with

a three-fold increase in overall lactate production. When oleate uptake was measured with or without glucose, we observed that glucose (5 mM, Fig. 3) induced a depression of oleate uptake. Thus, it appears from our

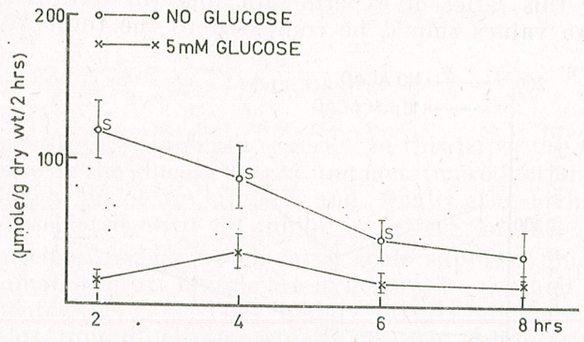

Fig. 3. — Oleate uptake by the isolated frog hearts, perfused with or without glucose and with 0.5 mM oleate. Each point represents the mean value from 6 to 10 hearts. All other details as for Fig. 2.

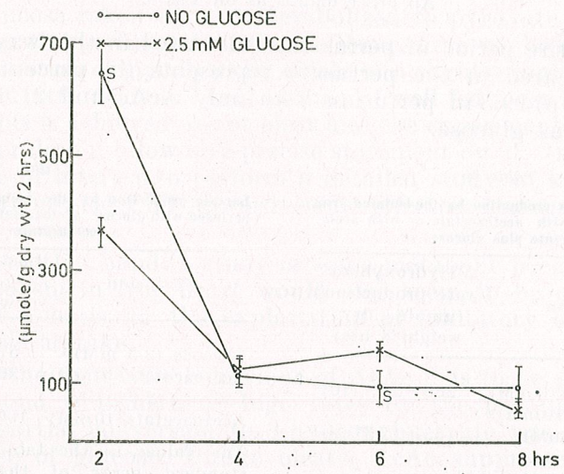

Fig. 4. — Acetoacetate uptake by the isolated frog hearts, perfused with or without glucose and with 10 mM acetoacetate. Each point represents the mean value from 9 to 10 hearts with standard error (vertical bars). S, as for Fig. 1. Animals of both sexes, weighing between 50 and 80 g., were caught in the middle of October, maintained in tanks with running tap water at 13°, with no food, and used for experiments during October and November. The perfusion was performed at 26°.

results that the competition between glucose and fatty acids as energetic fuels is solved in the isolated perfused heart in the favour of glucose.

Reciprocal effects of AcAc and glucose on their uptake. It is shown in Fig. 4 that AcAc uptake was inhibited by glucose for, at least, the

initial 3 hours of perfusion; then, a slight stimulation (at 6 hours) was observed, followed by no marked effect of glucose. The fraction of AcAc recovered as 3-hydroxybutyrate was also measured at the end of the perfusion for this series of experiments, and the data are recorded in table 3. These values should be compared to the total AcAc taken up

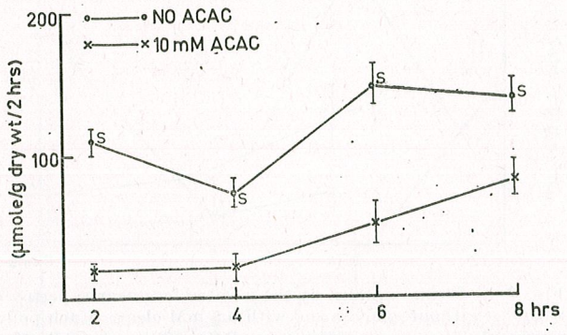

Fig. 5. — Glucose uptake by the isolated frog hearts, perfused with or without acetoacetate (AcAc) and with 2.5 mM glucose. All other details as for Fig. 4.

during the entire period of perfusion. Calculated in this way, 3-hydroxybutyrate appeared in the perfusate represents (in percentages of total AcAc taken up) 29.3 in perfusion with only AcAc and 21.6 in perfusion with AcAc plus glucose.

Table 3

3-Hydroxybutyrate production by the isolated frog hearts, perfused with acetoacetate or with acetoacetate plus glucose

Perfusion	3-Hydroxybutyrate production (µmole/g.dry weight/8 hrs)
Acetoacetate (10 mM)	283±25 (10)
Acetoacetate (10 mM)+ Glucose (2.5 mM)	155±17.5* (9)

The values in the table are mean ± standard error of the mean with the number of animals in parentheses. *The difference between the two groups is statistically significant at $P<0.001$. Experimental conditions as described in the legend to the Fig. 4.

Table 4

Lactate production by the isolated frog hearts, perfused with glucose, acetoacetate and glucose plus acetoacetate

Perfusion	Lactate production (µmole/g. dry weight/8 hrs)
Glucose (2.5 mM)	572±79 (15)
Acetoacetate (10 mM)	368±89* (10)
Glucose (2.5 mM) + Acetoacetate (10 mM)	1007±138* (9)

The values in the table are mean ± standard error of the mean with the number of animals in parentheses. *The difference from the values obtained in perfusion with only glucose is statistically significant at $P < 0.001$. Experimental conditions as described in the legend to the Fig. 4.

The effect of AcAc on glucose uptake is given in Fig. 5. A clear-cut inhibition of glucose uptake by AcAc was observed during the overall period of perfusion. The lactate concentration in perfusate, at the end of perfusion, was greater in the variant with AcAc plus glucose than in that with only glucose (Table 4). Lactate production in the perfusion with only AcAc occupied an intermediate position between these two values.

It appears from these results that, unlike oleate, ketone bodies inhibit glucose uptake winning, in this manner, the competition between them and glucose for being energetic fuels in the isolated perfused frog heart.

DISCUSSION

The most interesting data reported in this paper are the stimulatory effect of oleate on the glucose uptake and lactate production, the inhibitory effect of glucose on oleate uptake, and, finally, the high rates of AcAc utilization associated with its inhibitory effect on the glucose uptake.

The mechanisms by which fatty acids suppress glucose utilization by the mammalian heart muscle are in general known and experimentally well documented [2] [17]. They mainly involve an increase of intracellular concentration of citrate which, in turn, inhibits the activity of PFK. A reverse state, which would be characterized by a higher rate of glucose uptake in the presence than in the absence of fatty acids has not been reported so far for isolated organs. Such an effect is reported in this paper. It was associated with an increased lactate production, suggesting that the glucose taken up was metabolized to pyruvate which appeared as lactate in the perfusion medium. Hence, here we have to do with an actual stimulation of glycolysis by oleate at a slightly higher concentration than that of fatty acids in blood plasma of well fed frogs. The mechanism by which this is achieved is not clear and the experimental data reported in this paper do not allow any precise statement on it. In this connection it would be of interest to perform a detailed study of kinetic behaviour of frog-heart muscle PKF towards some effectors known for the enzyme from other sources.

On the other hand, a study of some metabolic peculiarities of fatty acid metabolism in frog heart would also seem to be of interest. This would offer a basis for the explanation of inhibitory effect of glucose on fatty acid uptake.

As to the metabolic behaviour of AcAc it is to be pointed out that this compound is utilized at high rates by the isolated perfused frog heart; its partial conversion to 3-hydroxybutyrate was not affected by the presence of glucose. Unlike oleate, AcAc suppressed glucose utilization leading simultaneously to an increase in lactate production. In this respect frog heart exhibits the same metabolic behaviour as mammalian heart does [24]. This suggests again that peculiarities of fatty acid and AcAc metabolism rather than some "unusual" properties of glycolytic regulatory enzymes (like PFK) in frog heart might be involved in the stimulation of glucose uptake by oleate.

Finally, since it has been reported that there is no ketogenesis in the liver of some amphibians, at least of those belonging to the genus *Rana*, the high rates of AcAc utilization, reported in this paper may not necessarily mean that AcAc is a natural substrate in *Rana*. If ingested by the animal, this compound may be activated by some enzymes of low specificity with respect to 3-ketoacids [7].

More detailed studies on the results observed here are in progress in our laboratory.

In conclusion, the data presented in this paper show that the isolated perfused frog heart does not behave in a similar manner to that of mammalian heart in respect with relationship between glucose and fatty acid metabolism.

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VARIATION OF THE ARGINASE ACTIVITY RELATED TO THE NUTRITION FACTOR IN THE PROCESS OF FISH ADAPTATION TO TEMPERATURE *

BY

AL. GABRIEL MARINESCU

The influence of the nutrition factor on the arginase activity in fish during the temperature adaptation was investigated.

Especially in 25° C acclimated fish it was recorded an outstanding increase of enzyme activity related to the advance of the starvation state (up 10—12-fold after 3 weeks of starvation).

In "food" group fish arginase has a compensatory type activity variation as against the adaptation temperature according to the Prechts type 3.

The variability of cell enzyme activity, depending on different environment factors and especially on the thermic factor was conclusively pointed out these last years [12] [22]. The involvement of the enzymic sequence in achieving the adaptation of fish to temperature is thought to be determining. Several experiments were undertaken in this respect in the aquatic vertebrates. Their results permitted to establish dependence relationships between cell enzyme activity and an environment factor [7—9] [15] [21] [22].

It is surprising that the study of a possible relationship between the enzyme and the nutrition factor has not been approached so far, though some investigations of this kind are already known in homeothermal vertebrates (Hock, 1967 ; Aumann et al., 1965 in [10] ; McDonald, 1965 in [10]). This is one of the reasons which induced us to study this relationship, the more so as previous investigations on the question of

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physiologic fish adaptation to temperature were pointing to such a possibility.

For the purposes of our working hypothesis we have chosen arginase (L-arginine amidinohydrolase, EC 3. 5. 3. 1), an enzyme of the hepatic cell, as its presence had previously been recorded in fish in a sure way [11] and, on the other hand, it is particularly interesting by its importance in the urogenetic cycle ([2] [4], Cohen, 1966, a.s.o.).

MATERIAL AND METHOD

Specimens of the species *Idus idus* L., collected from the coast lakes of the northern part of the Federal Republic of Germany, were used. Experiments were carried out in the zoophysiology laboratories of the Zoologic Institute of Kiel, under the guidance of Prof. H. Precht.

Fish groups were formed after a previous selection of the ichthyologic material, adapted to the laboratory conditions for at least 2 months (water quality and composition, light/darkness alternating for 12 hours, feeding on a concentrated product of known composition).

The experiment was performed according to the following variants:

a) optimization tests of enzyme activity in the determination conditions;

b) previous experiments;

c) determination of enzyme activity related to the nutrition state (in this case the adaptation temperature — AT according to Precht, 1949) was identical to the experiment one (ET) at 25° C;

d) the variation of arginase activity with various adaptation temperatures (25.15 and 5° C, respectively), when ET is always 25° C.

An original variant of the technique of arginase activity determination was used, which modifies the previously known ones (J. W. Campbell et al. [5]. J. B. Balinsky and E. Baldwin [2] for arginase determination; R. M. Archibald [1], G. W. Brown and P. P. Cohen [4] for urea determination). The modification made by us (in cooperation with one of the researchers of Kiel *) consists in determining urea with the Boeringer test much more economically and, hence, more accessibly. Moreover, the incubation medium of the raw enzymic material does not require special treatment. We mention that all tests carried out permitted the successful use of this variant of technique.

The enzymic material was prepared as follows: fish were sacrificed by decapitation and the removed hepatic tissue was introduced in an incubation medium (phosphate buffer solution 0.05 M, pH = 7.4, EDTA 1 mM) in a 1 : 11 ratio. Homogenization was carried out by means of an Ultra-Turrax in 4 stages of 30 sec. The respective sample was then introduced in the cooling centrifuge for 20 minutes at 15,000 × G, at a temperature of 2—3° C. The supernatant was immediately used to determine arginase activity.

Determinations of the protein content were performed in parallel for each separate sample, using to this end the Folin-Ciocâlteu method. These values were then used to calculate the so-called "specific activity of the enzyme" which is now considered to define in the most accurate way the magnitude of enzyme activity. This is expressed in μMol product per gram of fresh tissue and mg. per hour at a temperature of 25° C.

* H. Künnemann and Al. G. Marinescu, 1973, in print.

RESULTS OBTAINED

1. The dependence of arginase activity on the nutrition state

Figure 1 records the results of the experimental variant at 25° C (AT = ET), where specimens used by us had previously been adapted (or "acclimated", according to Hoar, 1967), to that temperature, and the experiment groups formed 2 main groups: on the one hand, fish fed on with the same amount of nutrient concentrate and, on the other hand, individuals submitted to progressive starvation.

Each point on the curves represents the average value for determinations carried out in 10—12 specimens (Fig. 1).

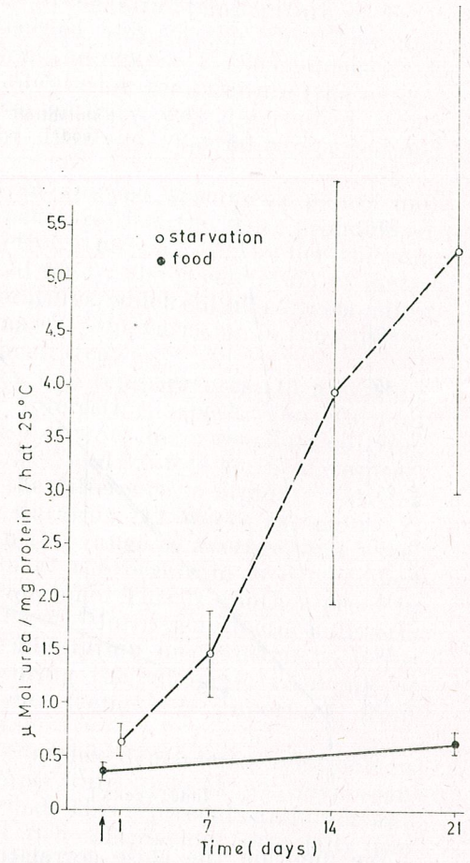

Fig. 1. — Arginase activity at 25° C (starvation and control).

2. Variation of arginase activity depending on the nutrition factor at different adaptation temperatures

Results recorded in the three experimental variants, corresponding to the three adaptation temperatures (25—25; 25—15 and 25—5°C), both in the "food" groups and in the starvation ones, are represented in figure 2, where average values are recorded for at least 10 specimens.

Results were calculated according to the model presented by J. W. Campbell et al. [5].

DISCUSSION OF RESULTS

As shown in the introductory part, we have chosen that enzyme of the hepatic cell of species *Idus idus* for several reasons. First, we have aimed at studying the changes in enzyme activity, in general, related to the nutrition factor and second, we intended to investigate an enzyme of the Krebs-Henseleit cycle, whose activity was supposed to respond to changes in the nutrition state. We mention that other experimental variants were carried out to the same effect, investigating the dependence of activity of other cell enzymes, both in the liver and in the muscle cell. These results will be recorded in a further communication.

An analysis of results shown in figure 1 points to an outstanding increase of enzyme activity related to the advance of the starvation state. It is worth underlining that this increase reaches important values (up to 10—12-fold), in the case of high temperature (25° C) after 3 weeks of starvation.

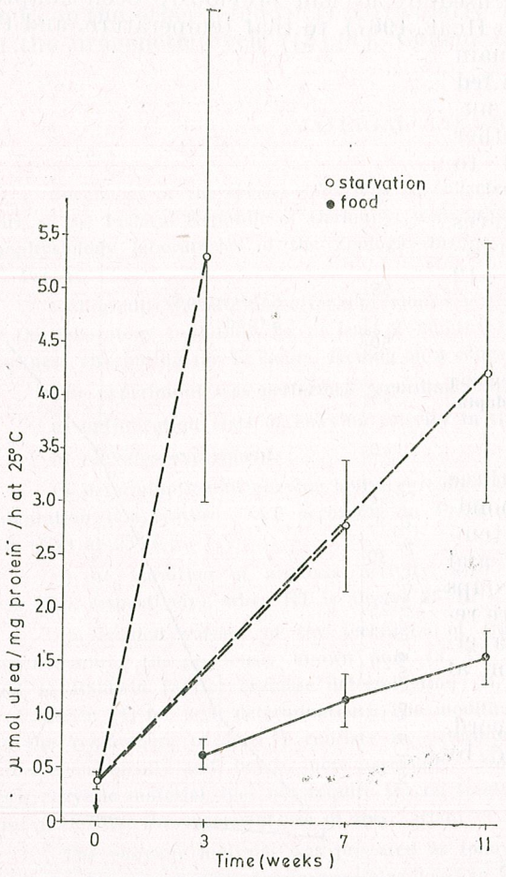

Fig. 2. — Arginase activity at three different adaptation temperatures.

We mention the close correlation between values obtained by us in the "food" groups (some 0.2—0.6 µMol/g. tissue/mg. protein/hour/at 25° C) and those pointed out by A. K. Huggins et al. [11] in Teleost fish. The absence of any indication in the speciality literature related to the dependence of enzyme activity on the nutrition state of fish, to which we could refer our results, prevents us in this stage of knowledge from setting forth an exhaustive explanation of the phenomenon. Data of subsequent researches should supplement our observations.

We think it necessary, however, to quote now the results obtained for other groups of vertebrates, viz. in mammals (A. Hock [10] and Muramatsu and Ashida, 1965 in [10]). In fish, dependence relations are

known between the nutrition state (starvation) and survival (Smith [23] [24]); the relationship between nutrition and oxygen consumption (Lindstedt [13]; Hoar [6]; Brett [3]; Pora et al. [18]; Picoș [16]; Picoș and Marinescu [17] a.s.o.); more recently, the relationship between the products of intermediary metabolism and the nutrition state (Pora and Precup [19]; Vellas [25]).

Figure 2 shows the average values of the activity of that enzyme in fish groups found during the adaptation process at temperatures of 15 and 5° C, as well as in the group adapted to the initial temperature of 25°C.

A comparison of the values recorded only in the "food" groups will evince an interesting fact, viz. that the enzyme has a compensatory type activity variation as against the adaptation temperature (the experiment temperature at which arginase activity was determined being constant — ET = 25° C) (see H. Precht, 1948 [20], partial compensation, type 3).

An analysis of values recorded in starved specimens points out that temperature (AT-adaptation temperature) has the most pronounced effect in the magnitude of enzyme activity only in the higher thermic zone (25° C). This is probably accounted for by the acceleration of metabolic processes in general at these temperatures, which entails corresponding changes in the reaction capacity of arginase — the variation in the activity of this enzyme being previously demonstrated.

By comparing values at the other two temperatures (15 and 5° C, respectively), we notice a particularly interesting aspect. The effect of starvation in the enzyme activity is also obvious here, reaching edifying values (an almost three-fold increase at 5° C and 2.5-fold at 15° C, respectively). Yet the order of magnitude of the difference in arginase activity obviously depends more on starvation duration (11 weeks as against 7) than on temperature. In other words, in this range of average, and especially lower, temperatures, the reaction of the organism is conditioned by the nutrition factor before the thermic one. This is another proof in support of an idea previously set forth by us (Marinescu, doctor's dissertation, 1972 [13]), according to which adaptation mechanisms to lower temperatures are, first of all, restructuring the energetic economy of those organisms, resulting in independence (sometimes important) from the limiting action of the thermic factor.

In cell enzyme activity as well, complex dependence relations to the action of environment factors (temperature and, as we have seen in certain cases, especially food) are recorded. This represents an important element for a more thorough study of fish adaptation (especially the physiologic one) to the temperature of the environment.

CONCLUSIONS

1. The results obtained point to an obvious involvment of the nutrition factor in the intensity of arginase activity in fish liver cell.

2. In the process of fish adaptation to temperature (species *Idus idus* L. in our case), the enzyme studied presents variable activity both as related to adaptation temperature (especially in the higher thermic

area) and, more particularly, as related to the magnitude and intensity of starvation.

3. A modified (original) variant was used to determine the activity of arginase in the hepatic cell of the fish class.

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THE INFLUENCE OF ADAPTATION TEMPERATURE ON THE BEHAVIOUR OF PARTIALLY PURIFIED L-ALANINE :2-OXOGLUTARATE AMINOTRANSFERASE FROM THE LIVER OF POND LOACH (*MISGURNUS FOSSILIS* L.)

BY

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L-alanine :2-oxoglutarate aminotransferase activity determined at three temperatures (2°C, 25° C and 37° C) presents higher values in total proteic extracts, obtained from the liver of cold-adapted pond loaches, as compared to those of warm-adapted fishes. By chromatography on Sephadex G-100 column (8 × 1.8 cm.), 5–30 times purified enzymatic preparations are obtained. By adaptation of fishes to low temperatures, $K_{m_{ala}}$ and $K_{m_{oxo}}$ decrease, thus enzyme affinity for 2-oxoglutarate and L-alanine increases. $K_{m_{oxo}}$ values are not affected by the temperature at which enzymatic reaction was performed.

Aminotransferases are very important enzymes for living organisms, having a special role in the biosynthesis and degradation of amino acids and therefore also of proteins. These enzymes form the connection between the metabolism of proteins, lipids and glucides by means of some oxo-acids representing substrates or products of transamination reactions.

L-alanine : 2-oxoglutarate aminotransferase (EC 2.6.1.2) which catalyses reversible reaction :

L-alanine + 2-oxoglutarate = pyruvate + L-glutamate was intensely studied in mammals [3] [9] [10] [11], fishes [5] [6] and plants [1] [2].

In a series of previous works [7] [8] we studied the modifications induced by the adaptation to low temperatures of pond loach in the activity of L-alanine : 2-oxoglutarate aminotransferase isoenzymes chromatographically separated from total proteic extract obtained from mus-

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cular tissue. By studying the variation of enzymatic reaction rate with the concentration of a substrate, under the conditions of maintaining constant the other substrate concentration, it is found that isoenzyme II, isolated from fishes adapted to 2° C, presents minimum K_m values for L-alanine and 2-oxoglutarate (thus maximum affinities) when enzymatic activity is determined at the adaptation temperature of animals. In the case of isoenzyme I, this rule is only respected in the case of K_{mala}. The total enzymatic activity is much higher in fishes adapted to 2° C, as compared with that recorded in fishes adapted to 25° C.

In this paper a similar study is presented, namely of the variation of K_m values for L-alanine and 2-oxoglutarate, in the case of the partially purified L-alanine : 2-oxoglutarate aminotransferase from the hepatic tissue of pond loaches adapted to 2° C and 25° C.

MATERIAL AND METHODS

Test animals — fishes (*Misgurnus fossilis* L.) — were obtained from the Greci pond of Romania. Adaptation was achieved in the laboratory at 2° C (cold) and at 25° C (warm) for 45 days.

Preparation of tissular homogenate. Hepatic tissue was homogenized in a Poter homogenizer, with cold K_2HPO_4/KH_2PO_4, 5×10^{-3} M pH 7.5 buffer solution (1g./10 ml.). The resulting homogenate was centrifuged at 10,000 r.p.m., for 15 minutes. The supernatant obtained was used for the partial purification of enzyme.

Partial purification of L-alanine :2-oxoglutarate aminotransferase was achieved by gel filtration on a Sephadex G-100 column (8 × 1.8 cm.) under the conditions described in a previous work [7].

Determination of enzymatic activity. The L-alanine :2-oxoglutarate aminotransferase activity was measured by colorimetric determination of the pyruvate formed as a result of enzymatic reaction, with 2,4-dinitrophenylhydrazine [9]. Enzymatic reaction was stopped after 30 minutes with 50% trichloracetic acid, while the pyruvate formed was transformed into a phenylhydrazone, which was colorimetrically dosed in alkaline medium at 540 nm. Enzymatic reaction was led at three temperatures : 2° C, 25° C and 37° C.

Determination of proteic concentration. The concentration of proteins in reaction medium was determined by the method described by Lowry et al. [4], using crystalline bovine serum albumin as the standard.

The rate of enzymatic reaction, catalysed by L-alanine :2-oxoglutarate aminotransferase was expressed in mμ moles pyruvate formed/mg. protein/minute/at 2° C, 25° C or 37° C. The results are expressed as substrate saturation curves (Michaelis-Menten). The Michaelis constant (K_m) and maximum velocity (V_{max}) for each substrate was determined.

RESULTS

In figure 1 we present the enzymatic activity, determined at three temperatures : 2° C, 25° C and 37° C, of total proteic extract obtained from the liver of fishes adapted to 2° C (hachured columns) and of those adapted to 25° C (white columns). It is ascertained that irrespective of the temperature at which the enzymatic reaction is carried out, the activity of L-alanine : 2-oxoglutarate aminotransferase from the proteic

extracts obtained from cold-adapted fishes is higher than that of extracts obtained from warm-adapted fishes. The fact is remarkable that at 2° C, the L-alanine : 2-oxoglutarate aminotransferase activity from total proteic extracts obtained from the liver of fishes adapted to 25° C is very small, slightly above the limits of the experimental error of the method.

The total proteic extracts were submitted to chromatographic separation on Sephadex G-100 columns (8 × 1.8 cm), previously equilibrated with a K_2HPO_4/KH_2PO_4, 5×10^{-3} M, pH 7.5 buffer solution. Protein elution was achieved with the same buffer solution, at a speed of 20 ml/hour, at a temperature of 4° C, 3 ml fractions being collected. The chromatograms obtained for L-alanine : 2-oxoglutarate aminotransferase isolated from fishes adapted to 25° C (A) and from fishes adapted to 2°C (B) are represented in figure 2. Purification factors varying between 5 and 30 are obtained. From No. 2 fractions, samples were taken on which all the Michaelis constants determinations were performed. In the case of these chromatograms it is likewise recorded that in the case of fishes adapted to 2° C, the profiles of elution curves, particularly in which enzymatic activity is determined at 2° C and 25C°, present higher activities.

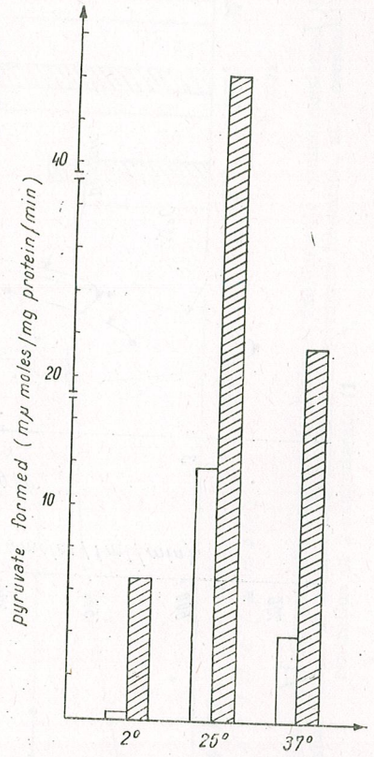

Fig. 1. — The activity of L-alanine : 2-oxoglutarate aminotransferase in the total proteic extract from liver of warm-adapted fishes (white columns) and cold-adapted fishes (hatched columns), studied at three assays temperatures : 2° C, 25° C and 37° C.

In figure 3 we present L-alanine saturation curves of partially purified L-alanine : 2-oxoglutarate aminotransferase from hepatic tissue of pond loaches adapted to 2° C and 25° C. From these saturation curves K_m were determined. Thus, in the case of cold-adapted fishes, when the determination of enzymatic activity was performed at 2° C, 25° C and 37° C, the following K_{mala} values are computed : 3.1 mM ; 5.4 mM and respectively 5.8 mM. It is noticed that the smallest Michaelis constant value, thus the highest L-alanine : 2-oxoglutarate aminotransferase affinity for L-alanine, is recorded at adaptation temperature. The further we move away from this adaptation temperature, the more the decrease of the enzyme affinity for its aminodonor substrate is emphasized. In the case of warm-adapted fishes, the following K_{mala} values are computed : 11.7 mM (2° C) ; 7.7 mM (25° C) and 6.6 mM (37° C). The slight enzyme affinity for L-alanine is recorded when the catalytic activity is dosed at 2° C.

In figure 4 we present the saturation curves in 2-oxoglutarate of L-alanine : 2-oxoglutarate aminotransferase, partially purified from the

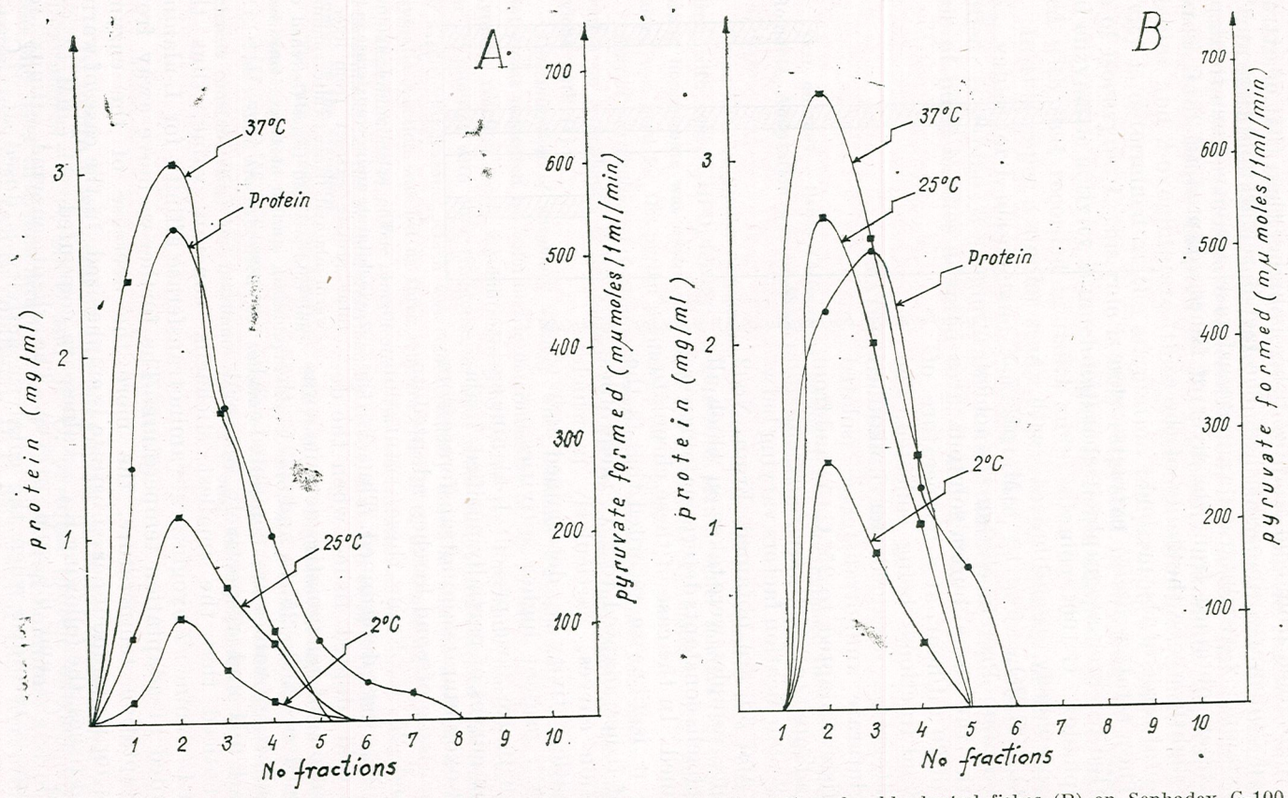

Fig. 2. — Gel filtration of total proteic extract from warm-adapted fishes (A) and cold-adapted fishes (B) on Sephadex G-100. Fractions of 3 ml each were collected. Enzymatic activity in each fraction was determined at 2° C, 25° C and 37° C.

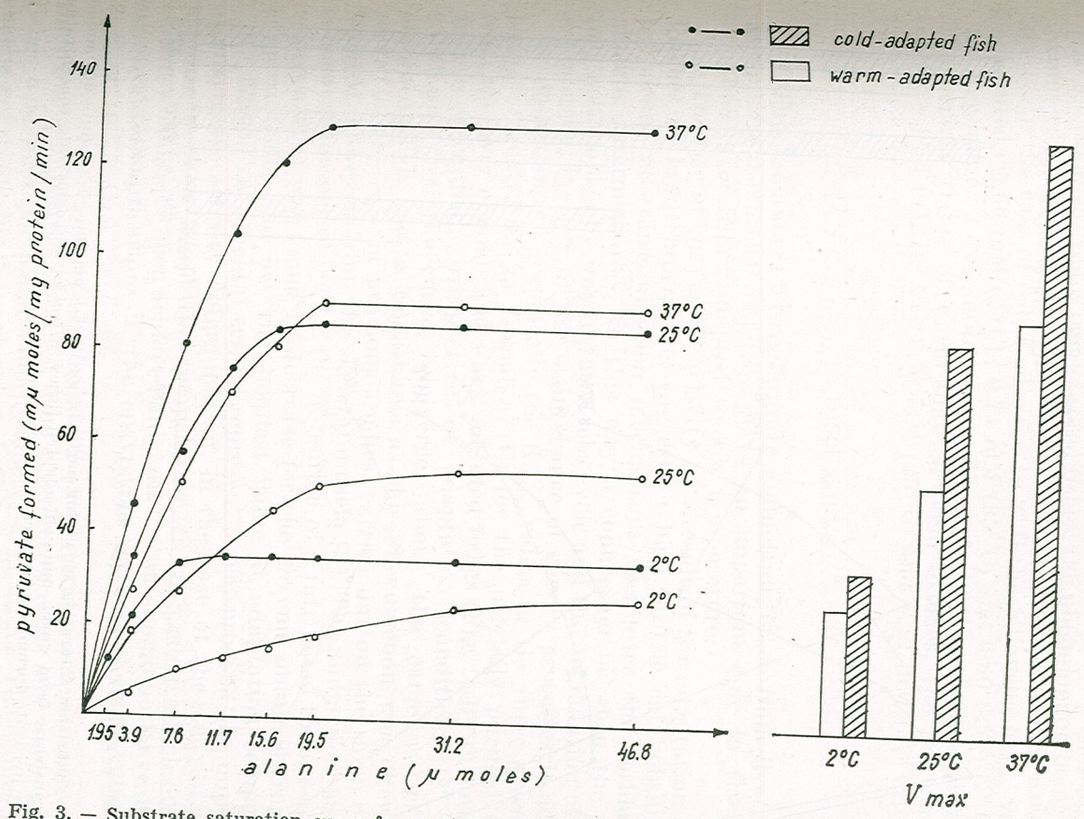

Fig. 3. — Substrate saturation curve for partially purified L-alanine :2-oxoglutarate aminotransferase from warm- and cold-adapted fishes. Effect of alanine concentration on pyruvate formation, at a constant concentration of 2-oxoglutarate (1 mM).

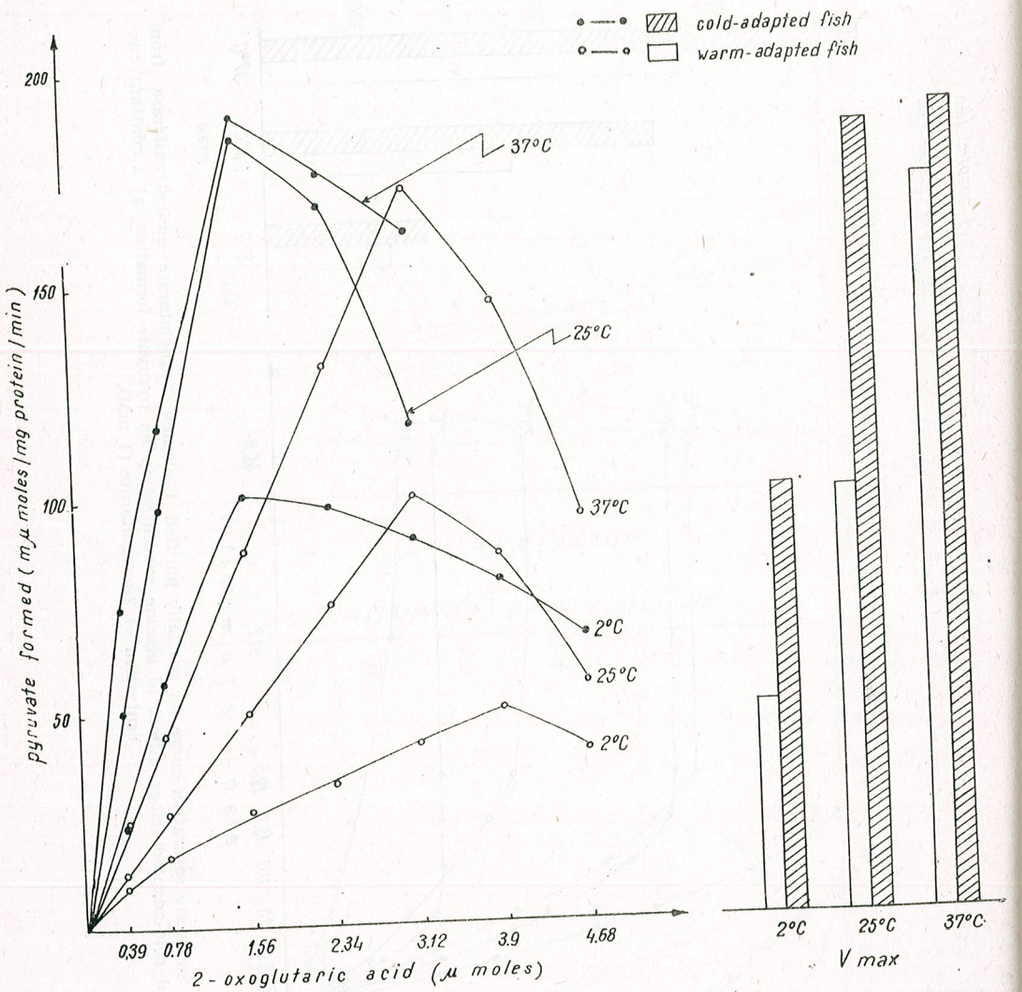

Fig. 4. — Substrate saturation curve for partially purified L-alanine : 2-oxoglutarate aminotransferase from warm- and cold-adapted fishes. Effect of 2-oxoglutarate concentration on pyruvate formation, at a constant concentration of alanine (39 mM).

liver of warm- and cold-adapted fishes. In this case, too, the maximum rate of reactions catalysed by the enzyme isolated from the liver of pond loaches adapted to 2° C acquire values higher to those obtained with the enzyme isolated from warm-adapted fishes, the phenomenon being strongly expressed in the case of the determination of these values at 2° C and 25° C. In the case of fishes adapted to 2° C the following $K_{m_{oxo}}$ values are computed : 0.66 mM (2° C) ; 0.74 mM (25° C) and 0.60 mM (37° C), while for L-alanine : 2-oxoglutarate aminotransferase, partially purified from the hepatic tissue of warm-adapted pond loaches, the following $K_{m_{oxo}}$ values : 1.56 mM (2° C) ; 1.56 mM (25° C) and 1.52 mM (37° C).

DISCUSSIONS

The L-alanine : 2-oxoglutarate aminotransferase from liver, as well as that from muscles [7] [8], plays an important role in the cellular metabolism of fishes adapted to low temperature.

The rise of enzymatic activity as a result of the adaptation of pond loaches to low temperature, suggests a high proteic catabolism, which by means of transamination mechanisms facilitates the formation of glucidic intermediaries. In this way a connection between the respiratory metabolism by way of the Krebs cycle and the degradation of nitrate compounds, in view of the maintenance of energy processes is achieved.

Representing graphically the $K_{m_{ala}}$ values at the three investigated temperatures (2° C, 25° C and 37° C) for the partially purified enzyme from the liver of cold- and warm-adapted fishes (Fig. 5, B) an interesting fact is recorded : irrespective of adaptation conditions (2° C and 25° C) at 37° C the curves converge, about the same values being obtained. This fact is remarkable as this phenomenon was likewise observed in the case of previous studies, carried out on the same enzyme, isolated, however, from the skeletal muscle [8]. The amino acid substrate concentration necessary for the obtention of a rate equal to half-maximal velocity (K_m) was found to decrease for the enzyme obtained from cold-adapted fishes and has the smallest value at adaptation temperature.

These experimental results represent a real proof of the importance of enzymatic activity determination, in the case of the study of poikilotherms, at their adaptation temperature. In the case in which our experiment would have been achieved only at 37° C we should not have detected significant modifications in the enzymatic activity of fishes adapted to 2° C or to 25° C.

The behaviour of L-alanine : 2-oxoglutarate aminotransferase to the oxoacid substrate obtained from the liver of cold- and warm-adapted fishes evidences particular kinetic aspects (Fig. 5, A). The values of Michaelis constants for 2-oxoglutarate are not affected by the temperature at which the enzymatic reaction is performed. The $K_{m_{oxo}}$ values in the case of L-alanine : 2-oxoglutarate aminotransferase isolated from the liver of cold-adapted fishes are smaller than those for the enzyme isolated from warm-adapted fishes, which evinces an increase of the affinity of this transaminase for its oxoacid substrate during the adaptation to low temperatures.

As in the case of other biological sources, in fishes, too, L-alanine : 2-oxoglutarate aminotransferase displays a greater affinity for its oxoacid substrate than to the amino acid one.

Of the two substrates which participate in the transamination reaction, the oxoacid substrate plays an important role in the regulation of enzymatic reaction. A more rapid saturation in the oxoacid substrate

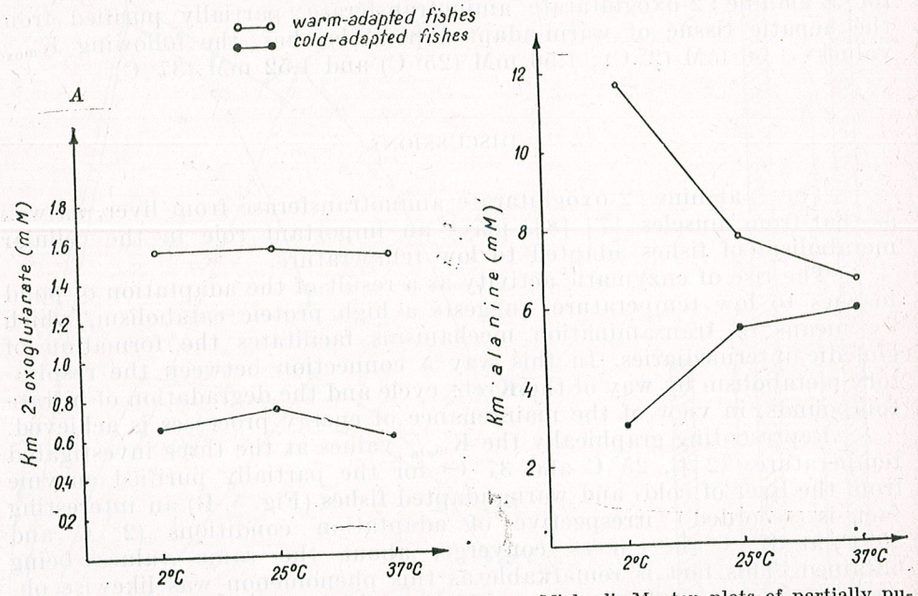

Fig. 5. — The Michaelis constants (K_m) derived from Michaelis-Menten plots of partially purified L-alanine :2-oxoglutarate aminotransferase for 2-oxoglutarate (A) and alanine (B), from cold- and warm-adapted fishes, obtained at three assays temperatures : 2° C, 25° C and 37° C

is recorded with the enzyme obtained from fishes adapted to 2° C (1.56 μmoles), as against the enzyme obtained from fishes adapted to 25° C (3.2—3.9 μmoles). Higher 2-oxoglutarate concentrations inhibit enzymatic reaction. The same phenomenon is recorded also in the case of L-alanine, with the difference that high aminodonor substrate concentrations do not affect the course of enzymatic reaction.

In conclusion, our data lead to the assumption that as a result of the adaptation to low temperatures or to high temperatures, modifications occur in the steric conformation of the enzyme. In the case of the adaptation to 2° C, these modifications lead to the formation of a catalytically more active state, in which the affinity for substrates is increased. It should not be lost sight of that the temperature itself at which enzymatic reaction is carried out may modify enzyme configuration, the relation between enzyme and substrate, enzyme-substrate complex formation or dissociation rate, a.s.o. Taking into account that these effects are produced parallelly in the case of the enzyme isolated from adapted or unadapted fishes, we may infer that the sole determining factor of the modifications detected by us is the adaptation temperature.

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THE ULTRASTRUCTURE OF NERVOUS GANGLIA AND OF NEUROHEMAL PERISYMPATHETIC NERVES IN *GRYLLOTALPA GRYLLOTALPA* L.

BY

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A study has been made on the ganglia of the ventral nervous chain and of correlated perisympathetic organs, in *Gryllotalpa gryllotalpa* L. References are made to the componence of neurons and of neuroglia types; perineurium, interneuronal gliocytes; neuroglial relationships: perineuronal glial sheath, interlacing of glial processes in neuronal incisurae, the presence of neuronal processes in intercellular spaces.

Mention is made of the active role of glial elements and of the neurilemma in the permanent transit in both directions between neurons and hemolymph. The ultrastructural aspect of perisympathetic nerves in the emergence and neurohemal regions is described.

The nervous system of orthopterous insects is an important field of investigation for specialists. A first group of concerns is represented by the elucidation of the structure and ultrastructure of neurons, neuroglial elements, interganglionic and peripheral nervous fibres [1], [7], [9] [16], [18]. Another series of works are referring to neurons with neurosecretion [4], [5] and to their reaction and that of neuroglia in experimental and regeneration conditions [6], [19], [20]. At last, particular attention has been paid to the neuroendocrine pathway (pars intercerebralis-corpora cardiaca-corpora allata), following up modifications that occur after its separation from the subesophagal ganglion in *Gryllus domesticus* [19]. On this occasion emphasis has been laid on the activity of the allatus II nerve and on its value as a neurohemal organ, adnex of the cardiac body. The same as other authors [22], we have pointed out the resemblance between this nerve and the perisympathetic organs described by Raabe in *Clitumnus extradentatus* [12].

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In the present paper, we shall approach the study of the structure and ultrastructure of the ganglia of the ventral nervous chain and of correlated perisympathetic organs in *Gryllotalpa gryllotalpa* L. with special reference to the neuroglial relations.

MATERIAL AND TECHNIQUE

Investigations were carried out in *Gryllotalpa gryllotalpa* L. on the ganglia of the ventral nervous chain and on perisympathetic nerves that present neurohemal differentiations on their course. Dissections were made directly in glutaraldehyde with sodium caccodylate buffer, at low temperature, and postfixation occurred in 2% osmium tetroxide solution buffered with sodium caccodylate. Fixation was also made directly in cold osmium tetroxide solution, as well as prefixation in glutaraldehyde with phosphate buffer, followed by postfixation with osmium tetroxide in phosphate buffer. The best results were obtained with the technique used in the former case. Dehydration occurred quickly and araldite was used for inclusion. Fine sections were stained for contrast, according to Reynolds (1962).

For optic microscopy, the material was treated according to current histologic techniques.

RESULTS

The ganglia of the ventral nervous chain in *Gryllotalpa* have at their periphery a neurilemma lined by a perineurium. There follows a layer of neurons and neuroglial elements, and a neuropil towards the interior.

The *neurilemma* appears under the microscope as thin, refringent and homogeneous. It is spreading continuously at the surface of the ganglionic nervous system, of peripheral nerves and of interganglionic connectives. Thus it plays the role of a selective barrier between the nervous system and the hemolymph.

Electron microscopic images present three zones making up the neurilemma: external — to the hemolymph —, middle and internal — formed of fibrils that recall the structure of collagen fibrils in vertebrates. The fibrils of the neurilemma are cross sectioned in the internal and external zones and longitudinally sectioned in the middle zone (Pl. I, Fig. 1). High magnification makes it easy to observe the periodicity of fibrils in the middle zone (Pl. II, Fig. 2 *a*).

The external zone loses the clear fibrillar aspect, becoming dense and amorphous. The described morphological characteristics are obvious in the neurilemma of somatic and sympathetic peripheral nerves and of interganglionic connectives.

The ganglion neurilemma has a less distinct middle zone, as longitudinal fibrils occur also in the external and internal zones, not only in the middle one. Moreover, the thickness of the neurilemma is not the same in all regions. The ganglion neurilemma is generally almost twice as thick as that of peripheral and perisympathetic nerves. Even the

ganglionic neurilemma varies in thickness throughout its area: it is narrower and denser in the areas of the ganglion where the neuron layer is thinner or absent.

The perineurium. It is a one-layer epithelium, in some places two-layer, placed between the neurilemma and the neuron layer. Perineurial cells are predominantly high, a few are globulous and flat; the latter have a denser cytoplasm. The perineurium is not equally thick throughout its length. Under the optic microscope, it appears that the perineurium becomes so thick in certain regions that the neurilemma comes into direct contact with the neurons. The electron microscopic images permit us to specify that in this region the contact between neurons does not occur, because of a very narrow space occupied by the processes of perineurial cells or of some glial interneuronal cells (Pl. III, Fig. 4). The remarkable mitochondrial wealth of the cytoplasm accounts for a denser aspect of these glial processes, as compared to the environing tissue. Mitochondria are generally elongated and have numerous cristae longitudinally or transversally oriented (Pl. II, fig. 2). The cytoplasm of perineurial cells also contain numerous polysomes and microtubules. The microtubules are tangentially disposed towards the neurilemma. Numerous inclusions are also present, more or less dense, with amorphous homogeneous aspect and variable shape and size. Some are confluent (Pl. II, Fig. 2 *c*). Inclusions of the same density, yet much smaller, can also be seen in the finest glial processes. Clear-cut isolated spaces or spaces that communicate with full inclusions with an amorphous content (Pl. II, Fig. 2 *d*) can frequently be observed. It is possible that these spaces be emptied inclusions. The same amorphous material is observed also in the cytoplasm of gliocytes, sometimes localized just near the nucleus. Because of these spaces, the cytoplasm of perineurial cells has an alveolar, spongy aspect. Spaces are numerous, of irregular shape and variable size. The cytoplasm cords that separate them are denser, due to the wealth of spheric and especially filamentous mitochondria.

The nucleus of perineurial cells is rich in chromatin and has an osmiophilic homogeneous nucleolus, shaped like a sphere. Huge masses and cords of chromatin are distributed both at the periphery of the nucleoplasm and in the close vicinity of the nucleolus. At this magnification, between the plasmalemmas of neighbouring perineurial cells, one may observe an intercytoplasmatic space which is often lacunary (Pl. II, Fig. 3).

Neurons are placed on 2—3 rows. Some are large, but numerous are medium and small. The former are considered motor neurons, the latter are association neurons [17]. Irrespective of their size, neurons are characterized by the presence of a little chromatic spheric or ovoid nucleus. The nucleolus appears as homogeneous, dense, osmiophilic and ovoidal. Electron microscopic images show in some neurons a nucleolar complex formed of a main nucleolus, with a thick nucleolonema, highly osmiophilic and with an irregular course. Near the nucleolonema are attached some small corpuscles as dense and osmiophilic as the main nucleolus, which may be considered satellite nucleoli. The same complex also includes a shapeless mass, less osmiophilic and more lax — probably the heterochromatin (Pl. IV, Fig. 6).

As concerns organites, the cytoplasm of all neurons presents numerous polysomes, neurotubules, frequent globulous or elongated mitochondria, lysosomes, cytolysosomes, dense bodies, smooth and granular endoplasmic reticulum (Pl. III, Fig. 4; Pl. IV, Fig. 6).

Neuroglial relations. From glial interneuronal cells, only the large polymorphous chromatic nuclei spread between neurons are visible under the optic microscope. They are difficultly seen as a whole in electron microscopic images. Yet their cytoplasm sends very fine processes through the neurons. Besides glial interneuronal cells, perineurial cells also establish close connections with the neurons, by sending fine processes both in the interneuronal space and in the space between the neurons and the perineurium. Glial interneuronal and perineurial cells therefore have very intimate morphological relations with the neurons. This certainly accounts for their particularly important role in the specific neuron activity: the receiving, processing and even transmitting of the neuron inflow. Glial and perineurial cells create the necessary trophic medium for neurons, selectively taking part — together with the neurilemma — in the transit of substances and ions permanently occurring between the neurons and the hemolymph. The same relation with glial cells could equally influence and favour the complex activity of cells with neurosecretion: to control the nervous inflow and to elaborate and remove a secretion product. The neuroglial association exists not only around the pericaryons but also around nerve terminals. Glial processes do not remain only in interneuronal spaces. In *Gryllotalpa*, the same as in the other insects, these processes penetrate in the deep incisures cut in the plasmalemma of the neuron perycarion. The processes are perfectly adapted to these incisures, so that the intercellular space, comprised between the neuronal and the glial plasmalemma, is distinguished with difficulty and only in certain regions. Submicroscopic processes are also sent in the interneuronal space by neurons, probably with a view to increasing their contact surface with a gliocyte.

The two types of processes are differentiated by their morphological characteristics: the processes of gliocytes are thin and numerous, those of neurons are bulky and separated by glial processes (Pl. I, Fig. 1; Pl. III, Fig. 4; Pl. IV, Fig. 5, 6). In the neuronal processes, the number of cytoplasmic inclusions is much lower than in the other part of the perikarion. Since neuronal processes are clearer, they can be easily distinguished from the glial ones, characterized by the great number of inclusions, especially mitochondria and vesicles with amorphous dense content. The amorphous content sometimes appears in close vicinity of the neuron plasmalemma (Pl. IV, Fig. 7). The same material of low density is often encountered among and in gliocytes. The crowding of glycogen aggregates (Pl. V, Fig. 7) and of mitochondria (Pl. VI, Fig. 8) around inclusions containing dense amorphous material is obvious and probably not due to change. For the time being, we cannot set forth an opinion on the chemical nature of this material, so often encountered in the ganglia of *Gryllotalpa*.

In the interneuronal space or among gliocytes, the presence of tracheocytes or only their processes could by observed. They are characterized by dense osmiophilic cytoplasm, full of very fine spaces which

account for its spongy aspect. The nucleus of the tracheocytes is polymorphous and the nucleolus has an osmiophilic component and a slightly osmiophilic one.

The conclusion may be drawn that the space between the neurons — which seems absent in optic microscopy — appears to be filled by neuronal, glial and tracheolar processes in the electron microscopic images. The abundant mitochondria in glial processes are an evidence of the essential role played by the cells of the perineurium and by perineuronal gliocytes in the course of neuron activity. The transit of the amorphous product from the neurilemma to the neurons or in the reverse direction seems to materialize the relationship of neurons with the hemolymph, by means of interneuronal gliocytes and of the perineurium. The transit to the neurons or from these to the hemolymph is not prevented but on the contrary favoured by the glial sheath, formed of 3—5 glial processes that cover the neuron.

The perisympathetic nerves. When analysing the ganglionic region, at the emergence point of the perisympathetic nerve (Pl. IV, Fig. 5; Pl. V, Fig. 7) the nerve neurilemma is observed only towards the hemolymph; in the contact area with the ganglion, the nerve is not separated by a neurilemma but by glial cells (Pl. V, Fig. 7), by their processes (Pl. IV, Fig. 5) and even by tracheocytes, placed between the gliocytes and the nerve. The gliocytes have a clear cytoplasm and their length is oriented to the emergence direction of the nerve. In their cytoplasm one may notice numerous free spaces or with a dense amorphous content, concentrating much glycogen around them (Pl. V, Fig. 7). Spaces with amorphous material are probably related with the depletion process of glycogen, which is massively deposited in the gliocytes cytoplasm. We assume, without being able to specify more so far, that the dense material could have a mucoprotein nature. Perineurial cells described in Pl. III, Fig. 3 differ from the type of gliocytes observed in Pl. V, Fig. 7, at the limit between the ganglion and the perisympathetic nerve. First of all, nuclei differ in that they are much more chromatic in the cells of Fig. 3, also characterized by the presence of a bulky, compact nucleolus. The nucleolus of clear gliocytes has a more lax nucleolonema. Mitochondria are much more numerous in the perineurial cells than in the described gliocytes. The latter contain more glycogen.

The neurohemal region presents axons of a variable size. Some have neurosecretion. In all axons there are neurotubules and mitochondria. Both in the axons with neurosecretion and in those without it, a high number of small synaptic vesicles are crowding at the terminal end or near the membrane: synaptoid and synaptic areas are sometimes observed on the same axon. There are axons dominated by elementary neurosecretion granules of small size, dense and homogeneous and especially clear, with a more dense core. There are also axons with larger granules, among which are also seen middle-sized and small granules. Irrespective of their size, neurosecretion granules are generally spheric, with the exception of some that seem to be confluent (Pl. VI, Fig. 8). The density of large granules is not the same: the less dense ones have a fine granular content — "structured" granules.

Around nervous fibres, with or without neurosecretion, are distributed the processes of periaxonal gliocytes, making up a glial periaxonal sheath, comprising one or several axons. On the site of synapses, the gliocyte processes are absent. Narrow junctions are established between glial processes. The length of junctions often recall "fasciae occludens".

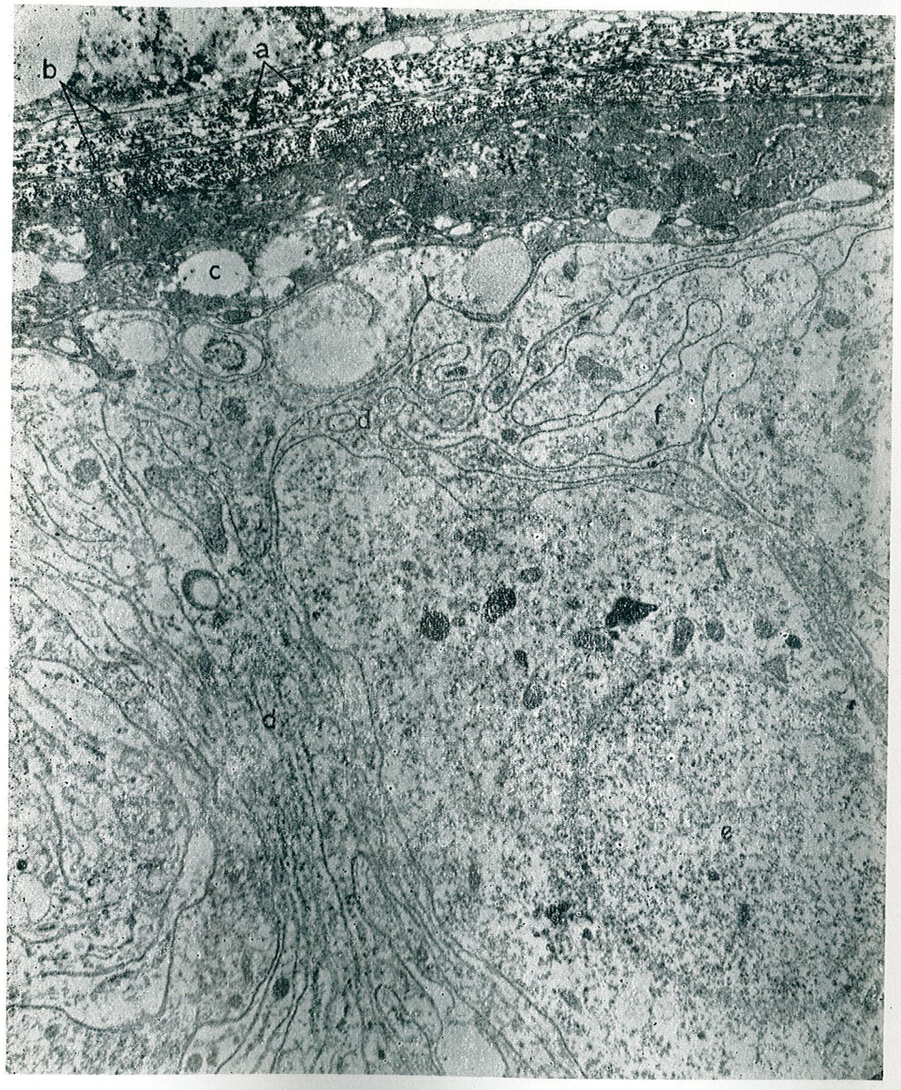
DISCUSSIONS AND CONCLUSIONS

Observations presented in this study refer more to relations existing between neuroglial interneuronal cells of the ganglia of the nervous chain in *Gryllotalpa gryllotalpa* L. We have also analysed the aspect of perisympathetic nerves corresponding to these ganglia, at their site of emergence and in the neurohemal region.

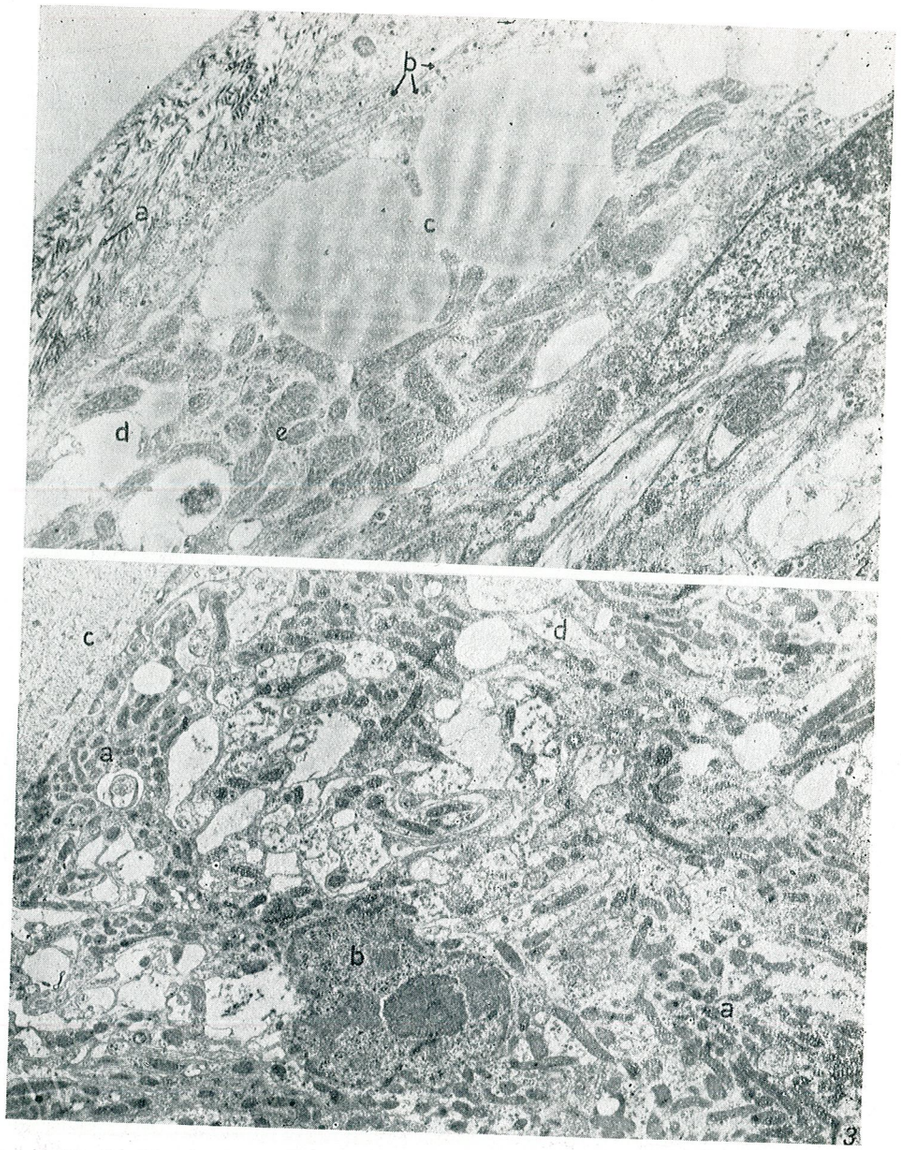
Many of the observations made by us corroborated in *Gryllotalpa* the results obtained by Hess [7] in *Periplaneta americana*. As against *Periplaneta*, where the glial sheath contains about 20 lamellae, in the neurons studied by us the glial sheath is much thinner (3—5 glial lamellae). The glial sheath has often interrupted lamellae, as mentioned also by Hess. Yet these interruptions are also due to the meandering course of lamellae which cannot be taken in on the same section in all their length. We cannot distinguish between types of neurons according to the size of organites, as mentioned in *Periplaneta*. The size of mitochondria varies, for instance, in the same neuron, but this is also due to the fact that they are not taken in under the same incidence in sections. Neurons have not seemed to differ by the aspect of mitochondria and of the other organites. In their cytoplasm are also revealed the elements of a rich vesiculo-tubular smooth endoplasmic reticulum and of a granular reticulum, scarce neurotubules, small dictyosomes, dense bodies of variable size, cytolysomes with lamellar or vesicular structure. Cytolysomes are much denser than mitochondria since some vesicles project towards the cytoplasm, acquiring a budding aspect.

As against *Periplaneta*, in the interneuronal space of *Gryllotalpa*, in addition to perineuronal glial sheaths there are submicroscopic processes of the neurons, aimed at increasing the contact surface with adjacent gliocytes. Therefore, in *Gryllotalpa*, the same as in other insects [7], [23], [24], neurons present plasmalemmal incisurae and neuronal expansions that are often very long and typical as concerns structure, shape and course. The interlacing of neurons and gliocytes is sometimes so perfect that the space between neighbouring plasmalemmas has to be revealed by special techniques. In molluscs, Mirolli et al. [8] have succeeded in demonstrating the existence of space by means of lantanum staining. Glial and neuronal processes in *Gryllotalpa* are, however, so different from the morphological standpoint that their interpenetration is doubtless.

The interesting studies carried out by Raabe [11] and Raabe et al. [13], [14], the same as those of N. de Bessé [2], [3] on the neurohemal perisympathetic nerves of some Orthoptera, record several types of neurosecretion fibres, based of the structure of secretion granules. In *Gryllotalpa*,

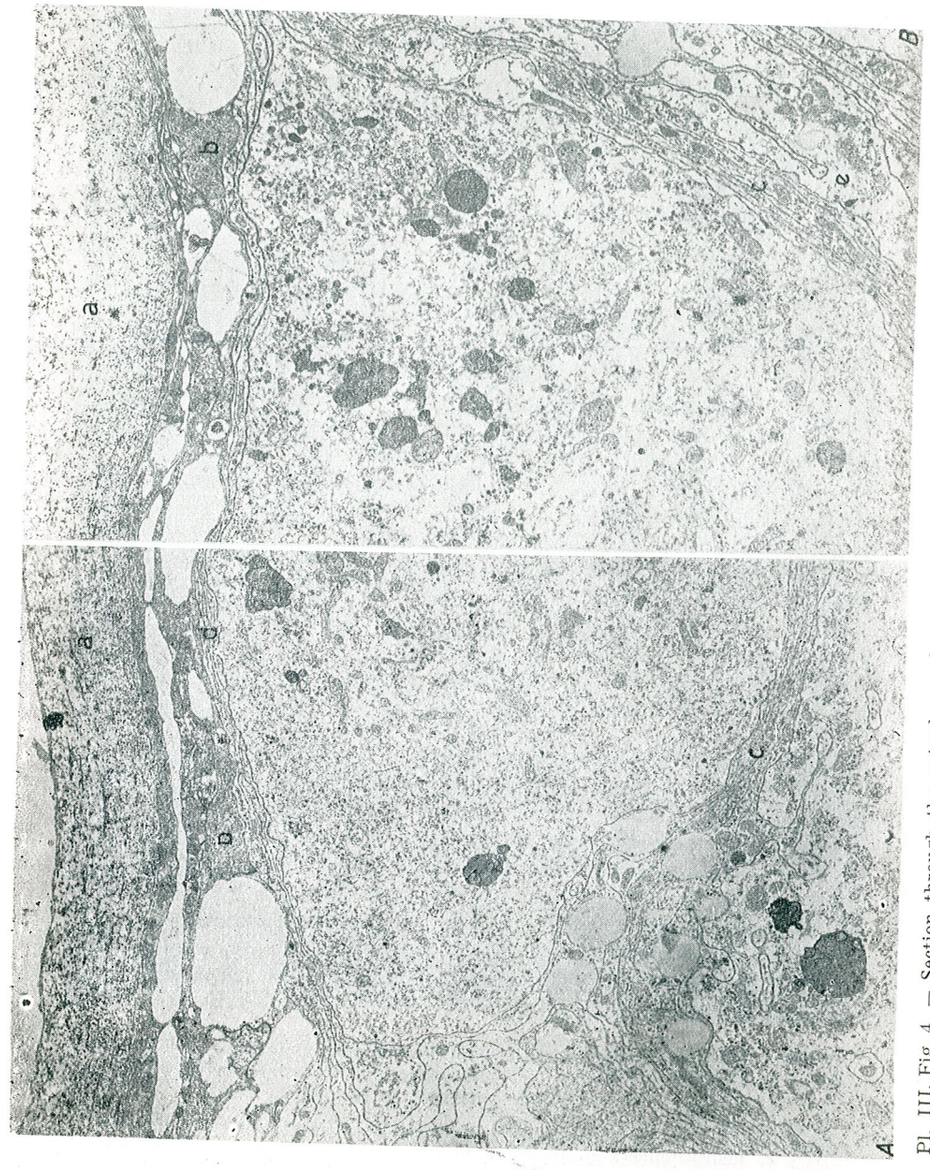


Pl. I, Fig. 1. — Nervous ganglion in *Gryllotalpa*; cortical zone, neurilemma; *a*, cross sectioned fibres; *b*, longitudinally sectioned fibres. Dense cytoplasm of perineurial cells, rich in mitochondria; *c*, intracytoplasmatic space; *d*, glial processes; *e*, neuron nucleus; *f*, bulky neuronal processes. × 5,150.

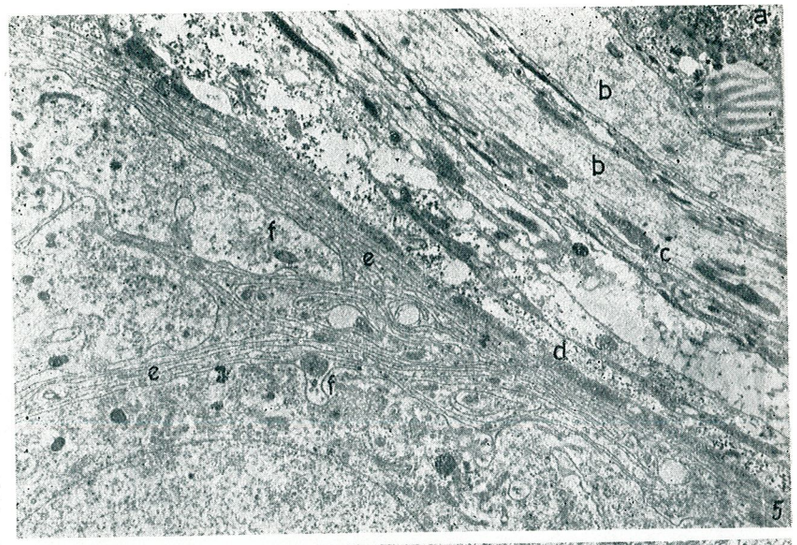


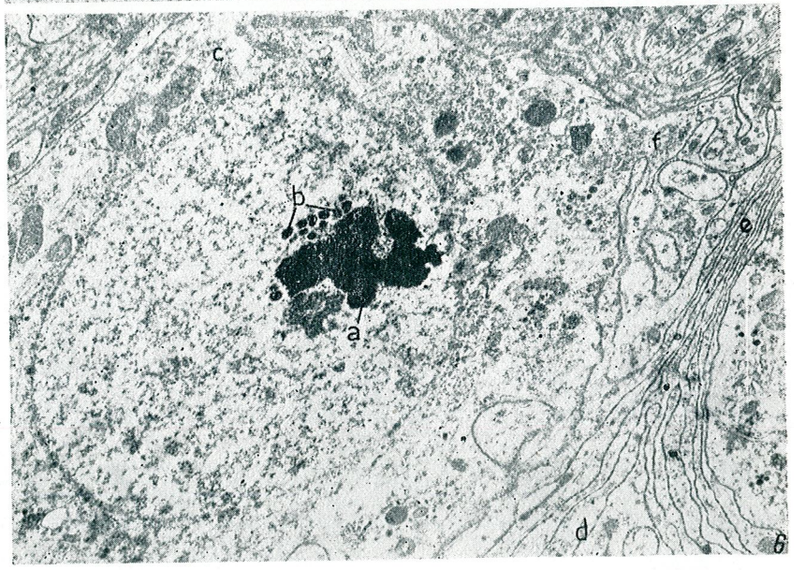
Pl. II, Fig. 2. — Peripheral zone of the perineurium. Neurilemma. *a*, periodic aspect of fibrills ; *b*, microtubules ; *c*, confluence of two inclusions containing amorphous and relatively dense material ; *d*, clear space, communicating with an inclusion with amorphous material ; *e*, filamentous mitochondria. × 9,450.

Fig. 3. — Perineurial cell, with vacuolized cytoplasm. *a*, denser zones of the cytoplasm with mitochondria ; *b*, nucleus of the perineurial cell ; *c*, neurilemma ; *d*, intercellular lacunar space. × 5,450.



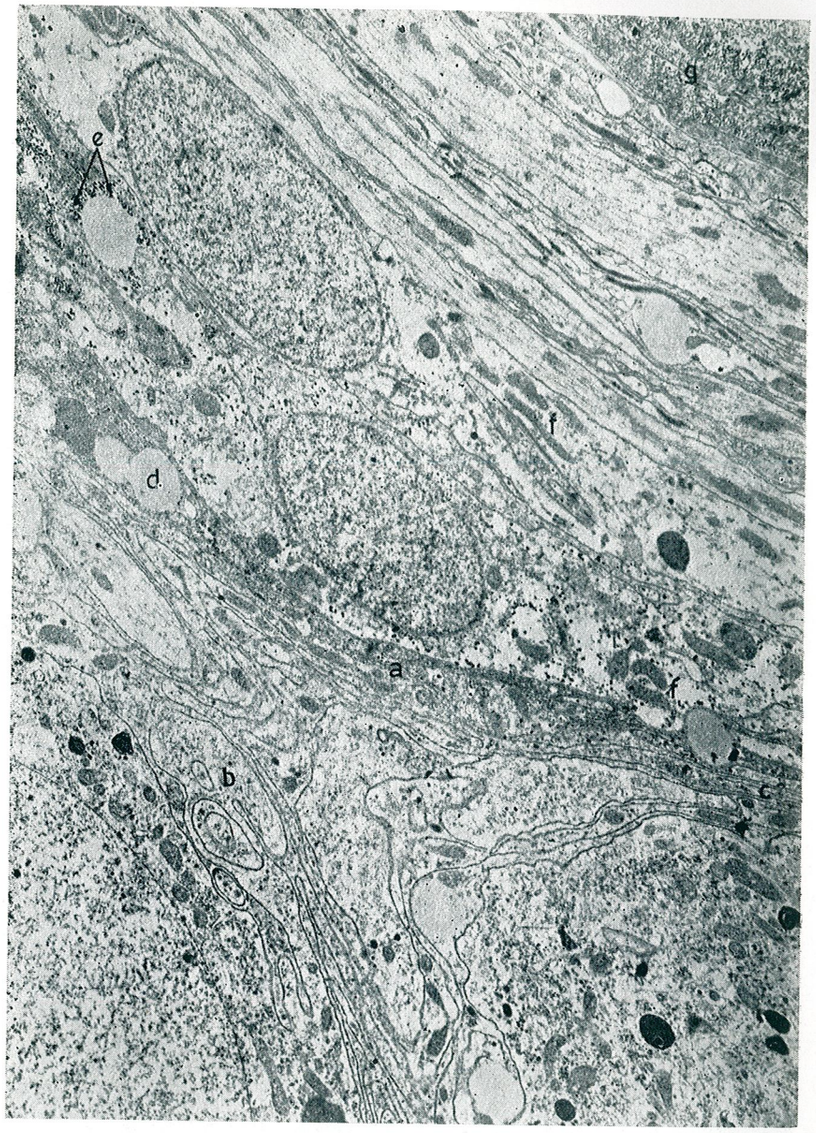
Pl. III, Fig. 4. — Section through the cytoplasm of a neuron at the ganglion periphery. *a*, neurilemma ; *b*, perineural layer ; *c*, interneuronal space ; *d*, perineuronal glial sheath, formed of several glial processes ; *e*, bulky neuronal processes with three parallel branches. Between branches, glial processes and a dense amorphous inclusion are seen. A × 3,960 ; B × 5,500.



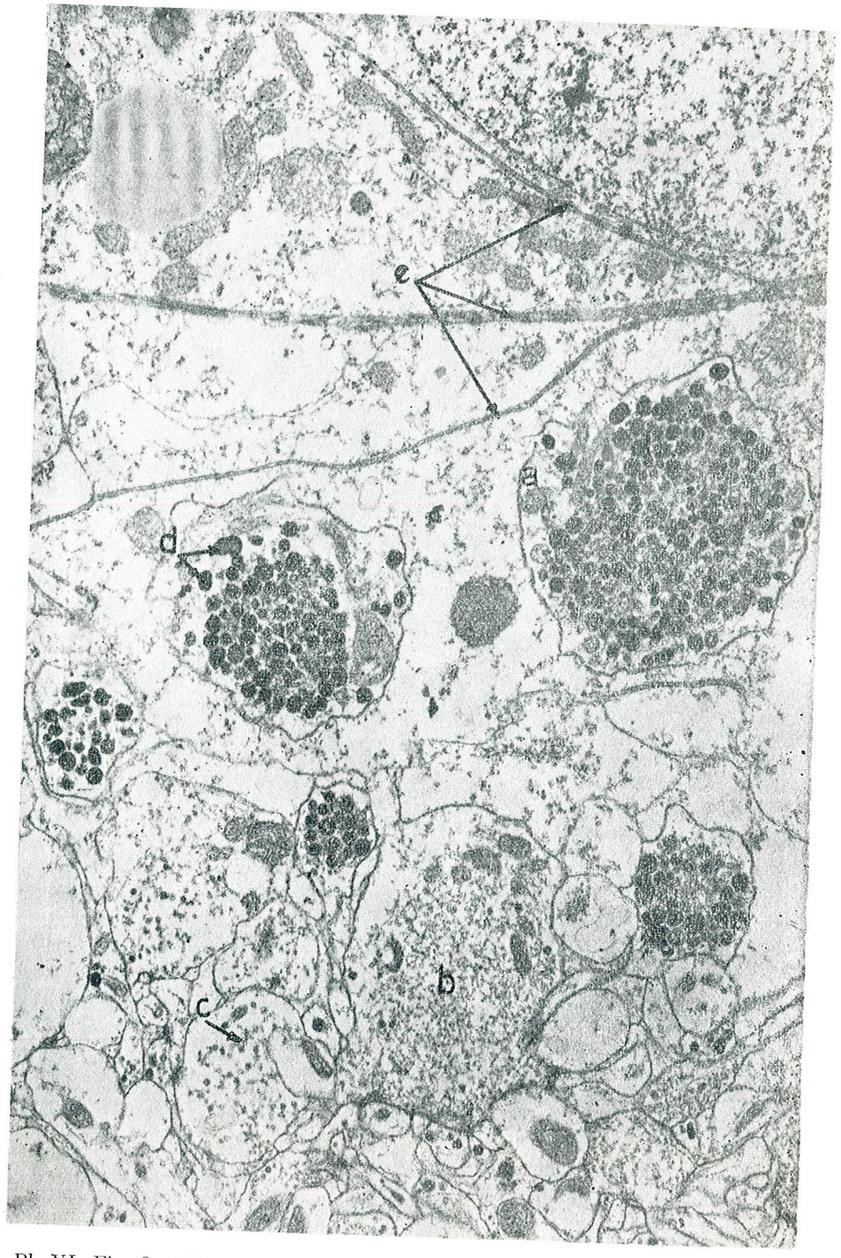


Pl. IV, Fig. 5. — Emergence of the peripheral nerve. *a*, neurilemma ; *b*, neurites with neurotubules ; *c*, mitochondria ; *d*, cytoplasm of periaxonal gliocytes ; *e*, perineuronal glial sheaths ; *f*, neuronal processes. × 7,750.

Fig. 6. — Association neuron. *a*, nucleolar complex ; *b*, satellite nucleoli ; *c*, neurotubules ; *d*, neuronal processes ; *e*, perineuronal glial sheath ; *f*, neuronal processes, bifurcate at its very origin, with meandering course. × 6,850.



Pl. V, Fig. 7. — Perisympathetic nerve and neighbouring ganglionic area. *a*, limit between the glial layer of the nerve and the ganglion ; *b*, neuronal processes ; *c*, glial sheath ; *d*, inclusions with dense amorphous material ; *e*, glycogen crowded around the inclusion with dense material, from the gliocyte cytoplasm ; *f*, filamentous mitochondria in the periaxonal glial cytoplasm ; *g*, the neurilemma of the perisympathetic nerve. × 4,450.



Pl. VI, Fig. 8. — Perisympathetic nerve in the neurohemal region. *a*, axon with neurosecretion; *b*, small synaptic vesicles; *c*, granules with dense core; *d*, granules with irregular outline and confluent granules; *e*, cytoplasmic processes of tracheocytes. × 6,240.

neurosecretion granules are dense and homogeneous, clear and with a dense core; some are large and less dense, with a fine granular content — "structured" granules.

For the time being, we shall not specify the types of fibres, according to the criterion of granule dimensions, especially as some fibres contain granules of different size and even structure. In their evolution, homogeneous and dense granules could pass through the stage of granules with a dense core, before being removed by exocytosis. Numerous granules with a more reduced size could by observed in the vicinity of the axon plasmalemma in the regions of maximum accumulation of small synaptic vesicles. We have also noticed granules that seemed to result from the confluence of others, or on the contrary some that looked like going to divide into finer granules.

We think, the same as other authors [10], [15] that the presence of neurosecretion granules among small synaptic vesicles at the terminal end of the nervous fibres proves that neurosecretion neurons play two functions, complexly correlated from a physiologic angle — that of a motor and of a secretory cell.

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P. RAICU, M. IONESCU-VARO, G. GANCEVICI et G. MOISESCU, *Celula* (La cellule), Editions de l'Académie de la République Socialiste de Roumanie, Bucarest, 1972, 343 pages.

Ces derniers temps on signale la parution de nombreux ouvrages concernant la structure et les fonctions de la cellule. Les nouveautés capables de changer nos conceptions sur la vie de la cellule se succèdent avec une rapidité qu'on peut comparer avec la rapidité qui a caractérisé l'apparition des données qui ont abouti, entre 1930 et 1950 à la découverte et à la mise en valeur de l'énergie nucléaire.

C'est un fait bien établi que les connaissances sur la cellule sont indispensables pour comprendre toutes les disciplines biologiques. Les progrès dans la biologie proprement dite, dans la médecine ou dans l'agriculture sont étroitement liés aux connaissances sur la cellule. Les phénomènes moléculaires et biochimiques dépendant des acides nucléiques sont d'une importance primordiale surtout pour la génétique.

Pour la bibliographie scientifique roumaine la parution du présent livre, une année après la Biologie cellulaire de I. Diculescu, D. Onicescu et L. Mischiu (1971) et deux années après le livre au même titre de M. Ionescu-Varo (1970), est une réalité à double signification : en premier lieu la nécessité d'être au courant avec les découvertes sur la structure et les fonctions de la cellule et en second lieu l'effort des auteurs de présenter aux chercheurs roumains les dernières nouveautés sur la cellule.

Le livre ci-dessus porte sur l'ultrastructure des organites cellulaires, la division, le matériel génétique de la cellule et des virus, l'enthalpie et l'entropie, le rôle des membranes dans l'organisation du flux énergétique de la cellule, la photosynthèse, la phosphorillation oxydative, l'énergétique de la biosynthèse des cellules autotrophes, le complément chromosomial et l'hérédité non chromosomiale, la recombinaison génétique chez les pro- et eucariotes et enfin la cytodifférenciation et le déterminisme embryologique.

L'énumération des titres, rend compte du grand nombre de découvertes récentes que ce livre renferme et surtout de son contenu dynamique sur la Vie cellulaire.

Les mécanismes d'autorégulation cellulaire sont spécialement mis en évidence dans tous les chapitres. L'information cellulaire tient un rôle capital dans la Vie cellulaire. Les auteurs insistent beaucoup sur ces informations de nature très différente afin de mieux expliquer l'autorégulation.

Nous nous permettons d'insister un peu sur quelques problèmes.

Les bases génétiques de l'hérédité constituent aujourd'hui la clef qui permet de comprendre la sélection et fait possible l'entrecroisement des races. Pour mieux présenter la replication de l'ADN viral les auteurs exposent l'hypothèse de *Cairns* (1964) et celle de *Gilbert* (1970). Ces nouvelles données offrent un aperçu clair sur l'entrecroisement des races, phénomène largement discuté dans la pratique agricole. L'ADN viral a permis d'avoir un modèle plus simple de la transmission de certains caractères parentals.

REV. ROUM. BIOL–ZOOLOGIE, TOME 18, Nº 4, p. 313–314, BUCAREST, 1973

Le processus de la biosynthèse protéique, qui depuis les recherches de *Jacob* et *Monod* a jeté une nouvelle lumière sur le mècanisme qui réside à la base de l'hérédité, est complété par les découvertes plus récentes de *Gruber* et *Campagne* (1966) et de *Clive* et *Bach* (1966). Une illustration adéquate accompagne le texte.

Après un exposé documenté on arrive à définir les gènes comme une chaîne polypeptidique et à les classifier en 4 catégories : structuraux, régulatoires, architecturaux et temporels.

L'énergétique cellulaire est liée aux données de la biochimie et de l'information. De l'énergie totale de la cellule (enthalpie) une certaine quantité est utilisée dans les réactions de synthèse et de transport, le reste servant au maintien de l'entropie cellulaire (système ouvert). De nombreux exemples sont donnés pour mettre en évidence l'utilisation de l'énergie pour différents types de cellules.

On démontre que la translocation ionique est à la base de tous les échanges à travers les membranes et qu'elle est la cause des variations bioélectriques de la cellule.

Un chapitre très condensé est consacré à la photosynthèse, l'un des plus importants phénomènes cellulaires pour entretenir la Vie sur la Terre.

Les recherches des deux dernières décennies sur les bactéries et les virus, ainsi que celles sur les organismes supérieurs, ont élucidé le concept classique de *Taylor* (1958) du « crossing-over » mitotique et intragénique. Un ample exposé est dédié à ce problème.

Enfin, nous tenons spécialement à mentionner le dernier chapitre sur la cytodifférenciation, phénomène encore assez mal connu. Il est la conséquence normale de la division cellulaire et de la formation de l'organisme pluricellulaire. On a pu démontrer que le facteur essentiel de la différenciation réside dans le noyau (expériences sur *Acetabularia*, sur *Xenopus*) et plus précisément dans les gènes du noyau. Les gènes existent dans toutes les cellules. Ils peuvent induire, par certaines substances, la nature des ébauches primordiales qui jouent le rôle de déterminants. Les expériences d'*Etienne Wolff* ont montré l'influence déterminante des tissus doubles (d'origine différente) sur la formation d'un organe final. Un court aperçu est donné sur la différenciation du sexe.

Le livre de P. Raicu, M. Ionescu-Varo, G. Gancevici et G. Moisescu représente un apport essentiel à la biologie roumaine. Sa parution récente marque un moment extrêmement important pour notre science qui est en cours de réorganiser les recherches biologiques. Paru aux Editions de l'Académie de la République Socialiste de Roumanie, ce livre se présente sous un aspect moderne et attractif.

Il doit se trouver dans la bibliothèque de tous les biologistes ainsi que de tous ceux qui dirigent le développement de la biologie et doivent connaître l'orientation actuelle de la Biologie mondiale.

Eugène A. Pora

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