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HETEROLEUCON HEARDI n. sp. FROM THE MEXICAN GULF

BY

MIHAI BĂCESCU

In the material from the Gulf of Mexico sent by Dr. Richard W. Heard, a number of species proved to be new to science. One of these, characterized by a huge siphon and a one-segmented uropodal endopodite, we dedicate to Heard, who collected it.

Diagnosis : long fine Cumacean showing a long upturned rostrum, prolonged by an enormous siphon. Antennule with a special tubercle on the second segment of the basis, ending in two enormous aesthetasks, nearly as long as the basis. Antenna with two large phanera having long hairs and a strong external plumose seta.

Description of manca ♀. Elegant body, impressive by the fineness of the abdomen and the exceptional length of all appendages. Tegument transparent, smooth, bearing rare and long hairs (Fig. 1 A, H). Cephalothorax slightly flattened laterally has a gentle crest and lacks the antennal notch.

A large siphon (longer than carapace) is prominent under pseudorostrum (Fig. 1 A) abruptly upturned and having long frontal hairs on its inferior margin. The eye lobe is weakly marked; the free thoracic segments suddenly decrease in width, the last one being hardly as thick as the first abdominal segment. Pleonites are cylindrical, the last one about twice as long as pleotelson; the latter is apically split (Fig. 1 H).

Antennula (Fig. 1 B) with a long basal segment, a bit longer than all the others, without aesthetasks; the middle segment shows a very typical subterminal tubercle on its internal and dorsal side bearing two special sensitive hairs (a cup-like peduncle and a long fibre reminding of choanocytes from spongia). The internal accessory one-segmented flagellum is very small, the big one is three-segmented, the last two segments bearing each an enormous aesthetask about as long as the remaining antennule.

Antenna (Fig. 1 C) has a strong two-segmented peduncle, the last segment ending in a large seta surrounded by long hairs and the basal segment in two thick phanera; it is densely haired in the external basal half and the rest of it bears long alternate hairs.

Mandible is provided with two teeth on pars incisiva and 4 lacinia wide at their basis, then geniculate and suddenly thinner (Fig. 1 D). The palp of maxilla ends in two long setae, the external one being twice as long. Third maxilliped (Fig. 1 E) has a basis shorter than the rest of seg-

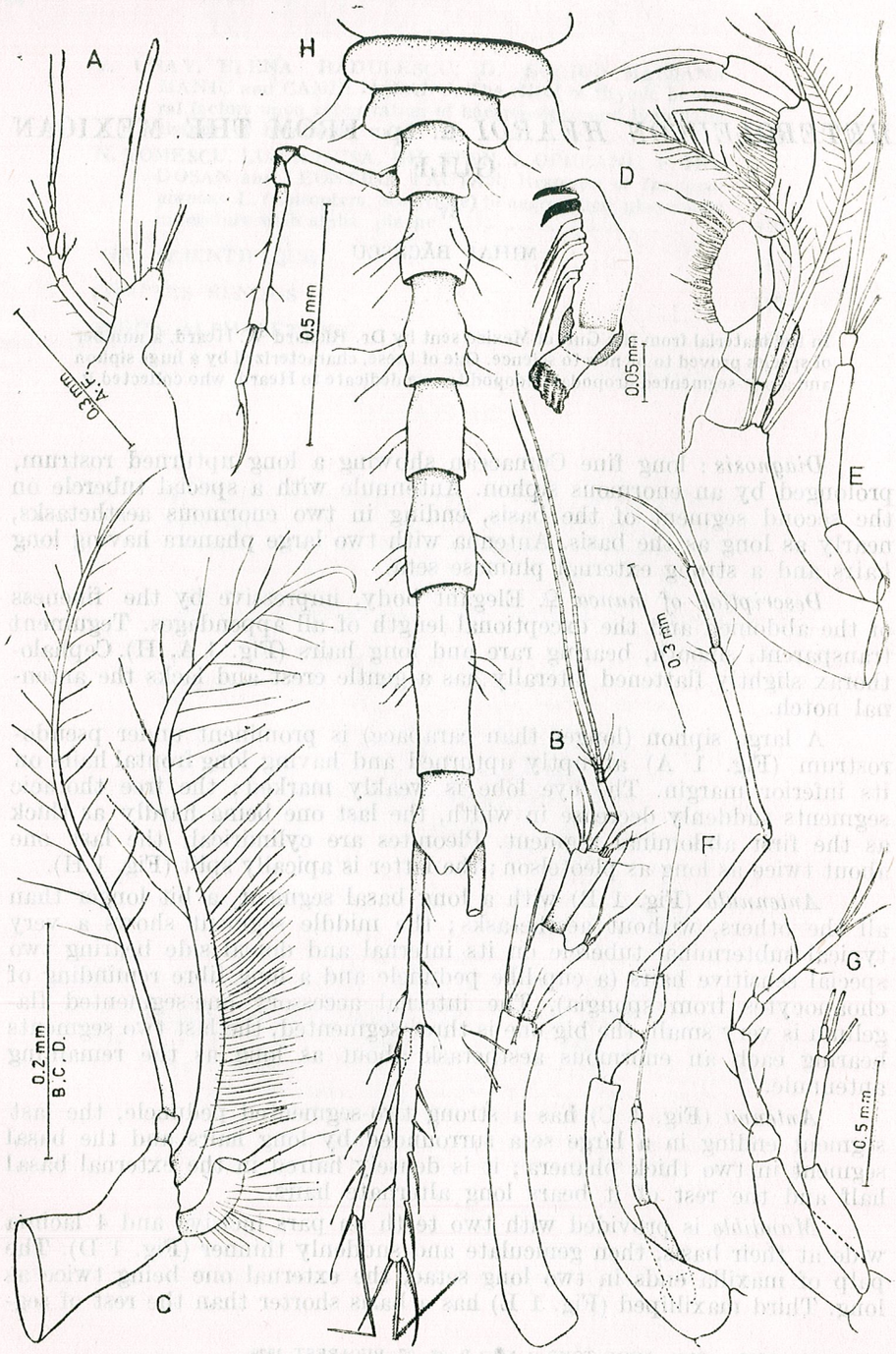

Fig. 1. — *Heteroleucon heardi* n. sp. (juv.).
A. Pseudorostrum, Antennule and the long siphon; B. Antennule, magnified; C, Antenna; D, Part of mandible; E, Maxilliped III; F, Peraeopod I; G, Peraeopod II; H, the posterior half of the Cumacean, dorsal view.

ments and bears two phanera resembling two big simple hairs and an immense long, haired one, like that of ischion. First peraeopod (Fig. 1 F) extremely long and fine, especially at the last three segments; basis as short as the following three articles together. It has an enormous fine dactylic claw. Second peraeopod (Fig. 1 G) is much shorter, about one half from the length of the first one; the other peraeods show the fineness and structure of the one represented in Fig. 1 H (the fourth one).

Uropods long and thin; the same fineness registered in their morphology as well as in the remaining appendages of this species; the basis of uropods being twice as long as pleotelson; the rami are fine as well: endopodite is one-segmented and clearly longer and more vigorous than exopodite (Fig. 1 H).

Material: 1 ♀ J (in fact in manca phase) of 2.5 mm (with siphon and uropods = 4 mm) originating in the Gulf of Mexico, South-Western Coast of U.S.A.; St. 28 F_2 leg. Dr. R. Heard, 31 Aug., 1976, 98 m.

Holotype no. 409 in the collection of "Gr. Antipa" Museum Bucharest.

Remarks. This species differs not only from all representatives of the genus *Heteroleucon*, but also from all Leuconidae by its enormous siphon which reminds of *Leucon syphonatus* Calman, but in *H. heardi* the siphon is upturned and very long. It is also characterized by its one-segmented endopodite of uropod and by the special structure of antenna. As we had only one specimen (this being not an adult) and as we could see clearly not even the structure of the basis of mandible, we confine ourselves for the moment to conside rour Cumacean as belonging to the afore-mentioned genus, although it also differs from *H. akaroënsis* Calman, the only species of this genus, by the extraordinary fineness of appendages (peraeopods, uropods and antennula).

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"Grigore Antipa" Museum of Natural History
Bucharest, Şoseaua Kiseleff 1

ON TWO NEW SPECIES OF *CAMPYLASPIS* (CUMACEA NANNASTACIDAE), THE DIAGNOSIS CRITERIA IN THE GENUS AND THE TWO NEW SUBGENERA: *SARSICUMA* AND *BACESCUA*

BY

ZARUI MURADIAN

Two new species, *Campylaspis vemae* and *C. menziesi* from the Atlantic and Pacific Panaman waters, are described. The author suggests as main determination criterion the aspect of maxilla and of maxillipeds I—II—III instead of carapace ornamentation. On this basis, 2 subgenera were established : *Sarsicuma* subgen. nov. and *Bacescua* subgen. nov.

The genus *Campylaspis*, one of the most abundant and interesting of the genera belonging to the order, is far from being exhaustively known. It would be enough to mention that 25 new species of *Campylaspis* were described as a result of the processing of the material collected only on two transects from the deep waters of the Atlantic [5]. That is why we were not surprised to find 2 new species in the material collected from the deep waters of the Atlantic and Pacific and offered for study by Prof. Dr. Doc. Mihai Băcescu. These are :

CAMPYLASPIS VEMAE sp. nov.

(Figs. 1 and 2 A—F)

Material : 1 ♀ M, Vema 15 — 21, 09°46,3′N 79°37,5′W, N. of Panama 938 m depth, 10 Nov., 1958.

Description of adult female. Carapace 2.5 mm in length, 1.25 mm in depth ; pseudorostrum very long, 1/6 of total length. Carapace and somites bear minute spines and sparse hairs. First two free peraeonites carry mediodorsal crests. Pleon, over 1/3 of total length (Fig. 1 A). Antennule (Fig. 2 A) with segments progressively smaller towards distal end. Mandible, maxillule, maxilliped I, maxilla as in Figs. 1 B-D and 2 B. Maxilliped II (Fig. 2 C) with a dactylus bearing 2 strong, subequal spines between which a much smaller one ; propodal spine longer than the dactylic ones. Maxilliped III (Fig. 1 E) with basis shorter than the rest of the appendage ; meros long and thin, as long as 1/2 of basis and twice as long as carpopodite ; propodite nearly as long as 1/3 of basis. Peraeopod I (Fig. 1 F) with basis somewhat longer than 2/5 of total length of the appendage segments, long and thin also shorter towards distal end. Pera-

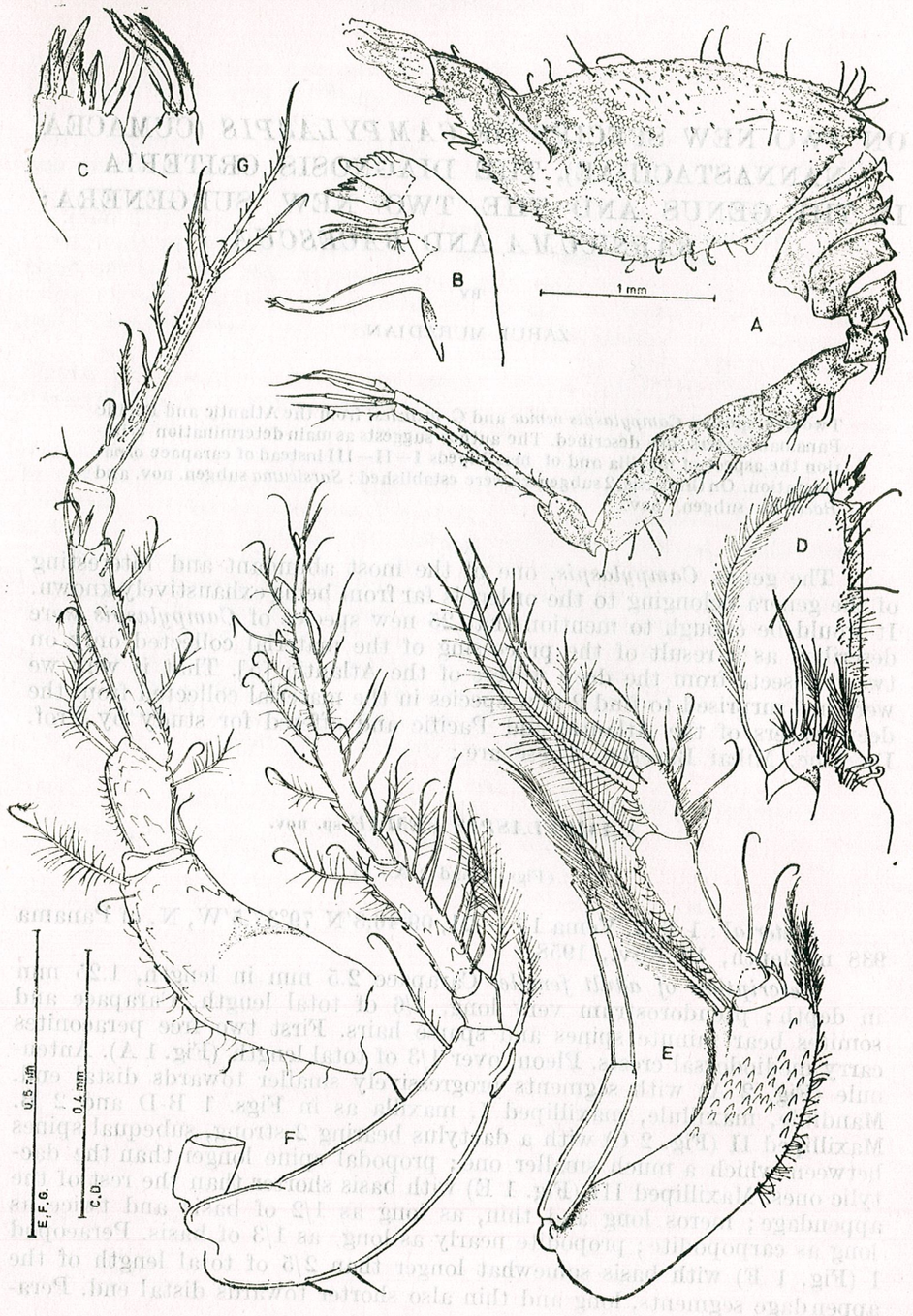

Fig. 1. — *Campylaspis vemae* sp. nov. ♀ ad. A, profile; B, mandible; C, maxillula; D, maxilliped I; E, maxiliped III; F, peraeopod I; G, peraeopod II.

eopod II (Fig. 1 G) with basis shorter than 1/3 of its total length; dactylopodite shorter than the combined carpopodite and propodite. Peraeopods III and V as in Figs. 2 D and E. Uropods (Fig. 2F) longer than the last 3 pleonites; peduncle 2 1/2 as long as endopodite, which is subequal with pleotelson.

Length : 5.6 mm

Holotype: ♀M, under no. 411 in the collections of "Grigore Antipa" Museum of Natural History.

Discussion and derivatio nominis. At first sight, the species resembles *C. aculeata* Jones and *C. rostellata* Jones, nearer to the latter, yet the differences noticed as maxilliped II, in the first place, do not allow assimilation with this species. Consequently, our specimen belongs to a new species named *vemae* after the name of the oceanic research vessel which brought such an abundant and interesting material for study.

CAMPYLASPIS MENZIESI sp. nov.

(Figs. 2 G—K and 3)

Material: 1 ♀ ov., St. 90 XI-Anton Bruun, 07°59′S 80°37′W, S. of Panama, 991 — 1015 m, Menzies Trawl, 14.X.1965; 1 ♀P, St. 94 XI-Anton Bruun, 08°21′S 81°25′W, S. of Panama, 1296—1317, Menzies Trawl, 14 Oct., 1965.

Description of adult female. Carapace with a lateral longitudinal depression, divided in two portions by a perpendicular carina. Hairs fine and sparse, spread particularly on the dorsal face of carapace (Fig. 3A). Antennula, maxillula, maxilla, maxilliped I as in Figs. 2 G-J and mandible as in Fig. 3 B. Maxilliped II (Fig. 2 K and k) with 3 dactylic spines of which the median is somewhat shorter than the lateral ones. Maxilliped III (Fig. 3 C) with a rather large meropodite as against the other segments. Peraeopod I (Fig. 3 D) with basis shorter than the rest of the appendage. Peraeopod II (Fig. 3 E) with basis about 1/3 of total appendage and dactylopodite longer than the combined carpopodite and propodite. Peraeopods III and V as in Figs. 3 F and G. Uropods (Fig. 3 H) approximately as long as 4 pleonites; endopodite slightly shorter than 1/2 of peduncle.

Length: 2.95 mm;

Holotype: ♀ ov., 07°59′S 80°37′W, 991 — 1015 m, under no. 412 in the collections of the "Grigore Antipa" Museum of Natural History.

Discussion and derivatio nominis. The species mostly resembles *C. reticulata* Gamô, as to the external appearance; they differ by the number of dactylic spines of maxilliped II and by uropodal ratios. We dedicate this species to late Prof. Dr. Robert Menzies to whom we are most grateful for the interesting material sent for study.

DISCUSSION ON THE CRITERIA FOR DIAGNOSIS IN THE GENUS

All keys published so far — Hale (1945), Jones (1974) — divide the genus in groups of species according to the ornamentation of carapace At first sight, it seems a convenient method to start determination from

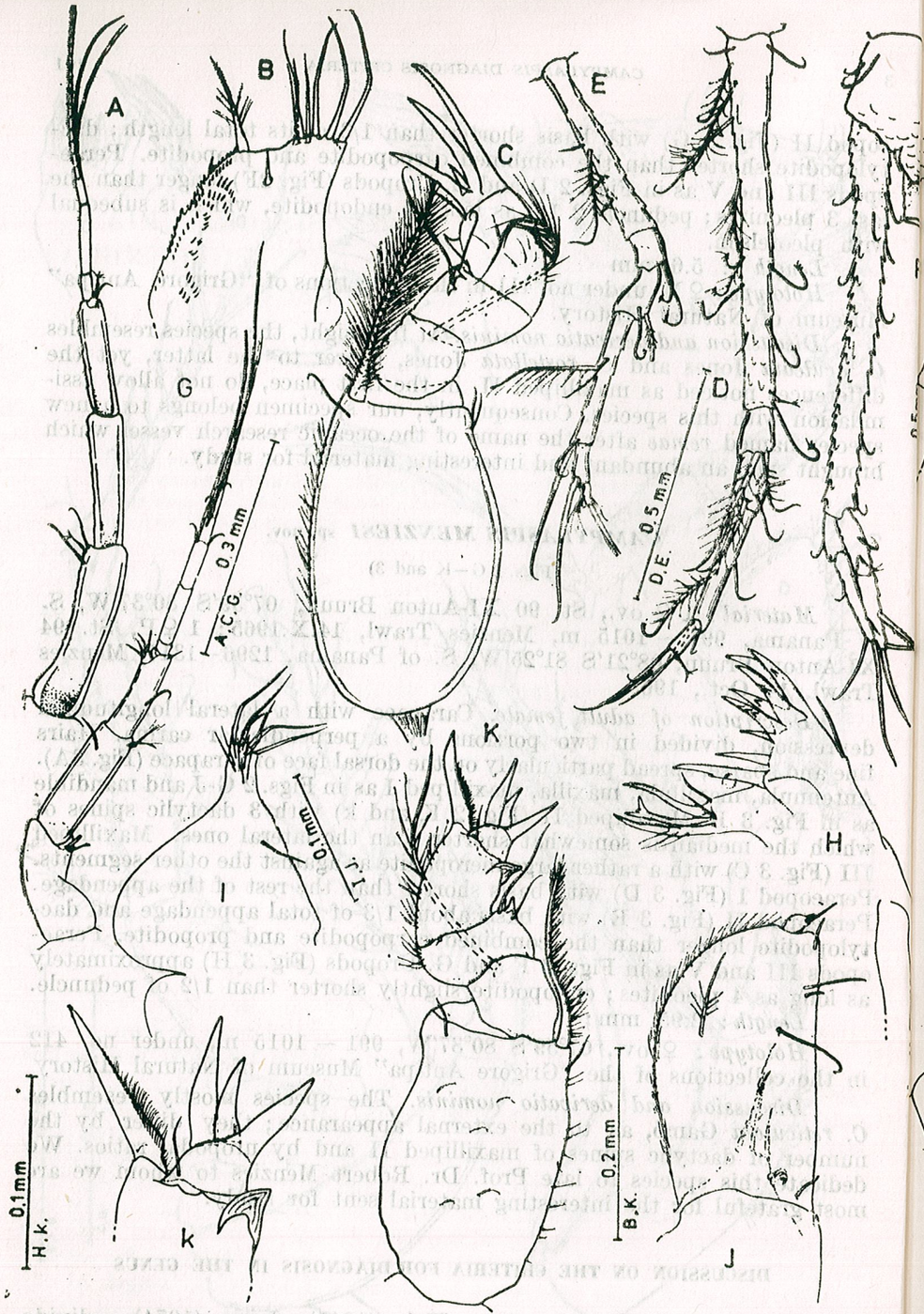

Fig. 2. — *Campylaspis vemae* sp. nov. ♀ ad., A, antennule; B, maxilla; C, maxilliped II; D, peraeopod III; E, peraeopod V; F, uropods. *Campylaspis menziesi* sp. nov. ♀ ad., G, antennule; H, maxillula; I, maxilla; J. maxilliped I; K and k, maxilliped II.

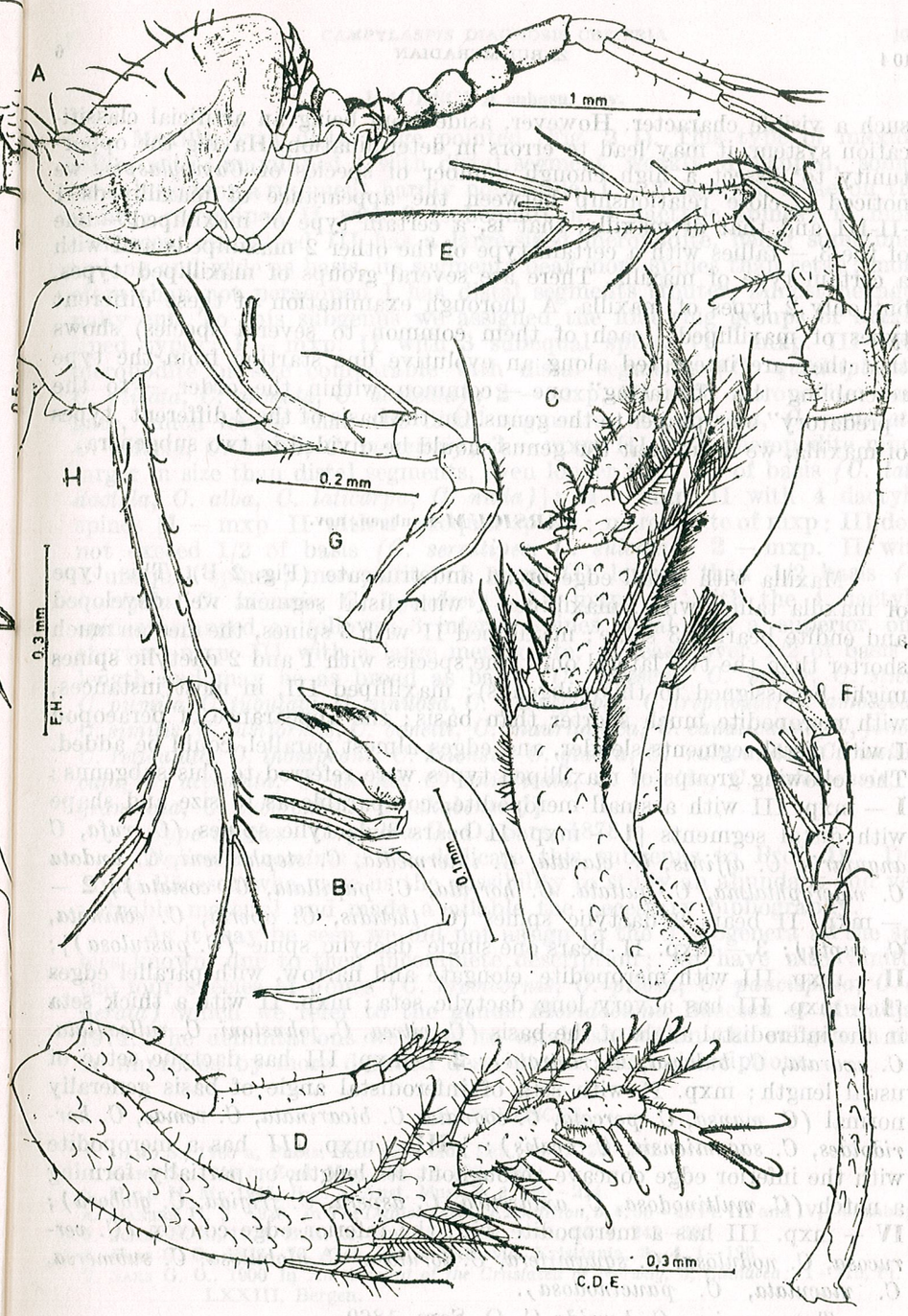

Fig. 3. — *Campylaspis menziesi* sp. nov. ♀ ad., A, profile; B, mandible; C, maxilliped III; D, peraeopod I; E, peraeopod II; F, peraeopod III; G, peraeopod V; H, uropods.

such a visible character. However, aside from being an artificial classification system, it may lead to errors in determination. Having the opportunity to dissect a high enough number of species of *Campylaspis*, we noticed a close relationship between the appearance of maxillipeds I -II-III and that of maxilla, that is, a certain type of maxilliped — one of the 3 — tallies with a certain type of the other 2 maxillipeds and with a certain type of maxilla. There are several groups of maxilliped types, but only 2 types of maxilla. A thorough examination of these different types of maxillipeds (each of them common to several species) shows that they are integrated along an evolutive line starting from the type resembling the "filtrating" one — common within the order — to the "predatory" one, proper to the genus. On the basis of the 2 different types of maxilla, we deem that the genus should be divided in two subgenera.

SARSICUMA subgen. nov.

Maxilla with distal edge broad and truncate (Fig. 2 B). This type of maxilla tallies with: maxilliped I with distal segment well developed and endite bearing 3 setae; maxilliped II with 3 spines, the median much shorter than the two lateral ones (the species with 1 and 2 dactylic spines might be assigned to this subgenus); maxilliped III, in most instances, with meropodite much shorter than basis; the appearance of peraeopod I with distal segments slender, and edges almost parallel, could be added. The following groups of maxilliped types were referred to this subgenus: **I** — mxp. III with a small meropodite, comparable as to size and shape with distal segments [**1** — mxp. II bears 3 dactylic spines *(C. rufa, C angularis, C. affinis, C. clavata, C. intermedia, C. stephenseni, C. undata C. macrophtalma, C. guttata. C. horrida, C. papillata, C. costata)*; **2** — — mxp. II bears 2 dactylic spines (*C. thetidis, C. aperta, C. echinata, C. rupta)*; **3** — mxp. II-bears one single dactylic spine *(C. pustulosa)*]; **II** — mxp. III with meropodite elongate and narrow, with parallel edges [**1** — mxp. III has a very long dactylic seta; mxp. II with a thick seta in the inferodistal angle of the basis *(C. vitrea, C. johnstoni, C. valleculata, C. exarata, C. bulbosa, C. rostrata)*; **2** — mxp. III has dactylic setae of usual length; mxp. II with seta of inferodistal angle of basis generally normal *(C. mansa, C. porcata, C. cognata, C. bicarinata, C. vemae, C. horridoides, C. sagamiensis, C. ovalis)*;] **III** — mxp. *III* has a meropodite with the inferior edge concave throughout its length, or partially forming a notch *(C. multinodosa, C. antarctica, C. aspera, C. frigida, C. globosa)*; **IV** — mxp. III has a meropodite with the inferior edge convex *(C. verrucosa, C. nodulosa, C. squamifera, C. torulosa, C. globulosa, C. submersa, C. maculata, C. paucinodosa)*.

Type species: C. horrida G. O. Sars, 1869

Derivatio nominis: We dedicate this subgenus to the late distinguished carcinologist Prof. G. O. Sars.

BACESCUA subgen. nov.

Maxilla with distal edge pointed (Fig. 2 I). This type of maxilla tallies with: maxilliped I with distal segment weakly developed, sometimes becoming a rudiment hardly noticeable, 1 — 2 rarely 3 setae on the endite; maxilliped II with 3 (subequal) or 4 dactylic spines; in most instances, maxilliped III has a large-sized meropodite, being sometimes as long and wide as basis, its segments bear more spines than setae; more often than not peraeopod I has distal segments stouter, almost hexagonally cut. To this subgenus we assigned the following groups of maxilliped types: **I** — mxp. II with 3 subequal spines [**1** — mxp. III with meropodite of size comparable with distal segments *(C. quadriplicata, C. plicata, C. arcuata, C. aculeata)*; **2** — mxp. III with meropodite elongate, much longer than the distal segments *(C. spinosa, C. rostellata, C. redacta, C. nitens, C. menziesi)*; **3** — mxp. III with meropodite much larger in size than distal segments, even longer than 1/2 of basis *(C. latidactyla, C. alba, C. laticarpa, C. nuda)*]; **II** — mxp. II with 4 dactylic spines [**1** — mxp. II with 4 unequal spines; meropodite of mxp: III does not exceed 1/2 of basis *(C. serratipes, C. sulcata)*; **2** — mxp. II with 4 unequal spines; meropodite of mxp. III longer than 1/2 basis *(C. laevigata, C. kiiensis, C. legendrei)*; **3** — mxp. II with the 4 dactylic spines arranged as follows; 3 inferior spines equal and a superior one, shorter; mxp. III with a large meropodite, at least over 1/2 of basis in length and may be as broad as basis *(C. bacescui, C. minor, C. sticta C. pumila, C. tubulata, C. sinuosa, C. paeneglaber, C. triplicata, C. amblyoda, C. similis, C. fusiformis, C. bonetti, C. mauritanica, C. canaliculata, C, jonesi, C. reticulata, C. thompsoni, C. kiiensis, C. glabra, C. rubicunda, C. unisul, cata, C. alveolata, C. striata, C. uniplicata, C. roscida, C. aulocoensis, C- granulata, C. tuberculata, C. akabensis)*].

Type species: C. glabra G. O. Sars, 1878

Derivatio nominis: We dedicate this subgenus to Prof. Dr. Doc. Mihai Băcescu who gave us the possibility to study an abundant and very valuable material and made available the necessary bibliography.

As it may be seen we did not assign to the 2 subgenera all the species known, due to their incomplete description; we have also omitted the four species of Jones *(C. brevicornis, C. pilosa, C. paucispina, C. caperata)* which we refer to the genus *Floridocuma* Băcescu et Muradian, 1974. The delimitations we tried to establish within the subgenera may be improved by more detailed descriptions and redescriptions.

REFERENCES

1. Gamô S., 1960 a, Publs. Seto mar. biol. Lab., **8**, *1*, 153—161.
2. Gamô S., 1960 b, Zool. Mag., **69**, *12*, 369—387.
3. Hale H. M., 1945, Rec. S. Aust. Mus., **8**, *2*, 180—214.
4. Hansen H. J. 1920, In *The Danish Ingolf-Expedition*, **3**, *6*, 36—48 Pl. III and IV, Copenhagen.
5. Jones N. S., 1974, Bull. Br. Mus. nat. Hist. Zool., **27**, *6*, 249—300
6. Sars G. O., 1878—79, Arch. Math. Naturvid. Kristiania, **3—4**, 1—196
7. Sars G. O., 1900 In *An Account of the Crustacea of Norway*, **3**, *Cumacea;* 1—115, Pl. I — LXXIII, Bergen.

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MODIFICATIONS MORPHOLOGIQUES DE L'ORGANE TRICHOBOTHRIAL DURANT L'ONTOGÉNIE DES ORIBATES (*ACARI, ORIBATEI*)

PAR

MAGDA CĂLUGĂR et N. VASILIU

The authors present the modifications occurred in the trichobothrial organ during the ontogenesis of two Oribatids species.
In *Nothrus palustris*, this organ appears only within the protonympha, at the following stages a completion of this structure takes place.
In *Tectocepheus velatus*, the organ is completely formed starting from the larva. At the following stages only a stepwise chitinisation is observed.

La trichobothrie, l'organe de sens caractéristique des arachnides atteint le plus haut degré de complexité et de diversité morphologique chez les oribates. Dans la structure de cet organe on distingue : un poil sensitif (le sensillus), une coupe chitineuse invaginée (la bothridie) et une membrane élastique (membrane articulaire). Caractéristique de certaines oribates macropylines (*Phthiracaroidea* Grandj., 1954 et *Euphthiracaroides* Grandj., 1967 ; *Nothridae* Berl., 1896) est la présence des trachées variées comme dimension, nombre et aspect qui s'ouvrent dans la portion interne de la bothridie.

La présente Note étudie les modifications morphologiques subies par l'organe trichobothrial en ontogénie des espèces *Nothrus palustris*, C. L. Koch, 1839 et *Tectocepheus velatus* (Michael, 1880).

RÉSULTATS

Développement ontogénétique de la trichobothrie chez Nothrus palustris. Caractères dimensionnels. L'analyse de l'indice de croissance relative montre que le rythme de croissance du sensillus est plus accentué chez la protonymphe et la deutonymphe ; la bothridie présente une croissance graduelle dès la protonymphe à l'adulte et les trachées ont un rythme de croissance semblable à celui du sensillus (tabl. 1, fig. 1 *a*).

Caractères morphologiques. Chez la larve (fig. 2*a* ; *a'*) l'organe trichobothrial est représenté par un simple poil effilé à la partie basale enfoncée dans l'épaississement du tégument.

Chez la protonymphe (fig. 2*b* ; *b'*) on observe que l'organe trichobothrial est déjà constitué, comme chez l'adulte, par sensillus, bothridie, membrane articulaire et trachées. Le sensillus filiforme allongé, rarement, barbulé, orienté latéralement, présente 3 courbures fixes : une à l'extérieur

REV. ROUM. BIOL. – BIOL. ANIM., TOME 24, Nº 2, P. 107–112, BUCAREST, 1979

Tableau 1

Dimensions, en microns, de la longueur du corps et des éléments de la trichobothrie chez les stases de *Nothrus palustris*, exprimées par valeurs absolues et relatives

Stases	Valeurs	Longueur			
		Corps	Sensillus	Bothridie	Trachée
Larve	absolue	500	17	2,5	—
	— % —	—	3,40	—	—
Protonymphe	absolue	634	285	23	15
	— % —	—	44,95	3,63	2,36
Deutonymphe	absolue	793	342	38	17
	— % —	—	43,12	4,79	2,14
Tritonymphe	absolue	1 110	372	53	17
	— % —	—	33,51	4,78	1,53
Adulte	absolue	1 126	388	62	22
	— % —	—	34,45	5,50	1,95

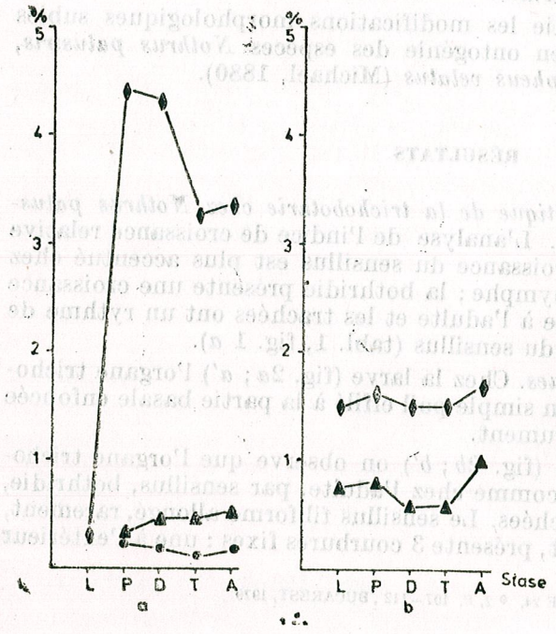

Fig. 1. — Accroissement relatif de l'organe trichobothrial durant l'ontogénie des espèces;
a, *Nothrus palustris*: *b*, *Tectocepheus velatus*.

Explication ♦ *Sensillus*: ▲ Bothridie; ● Trachée

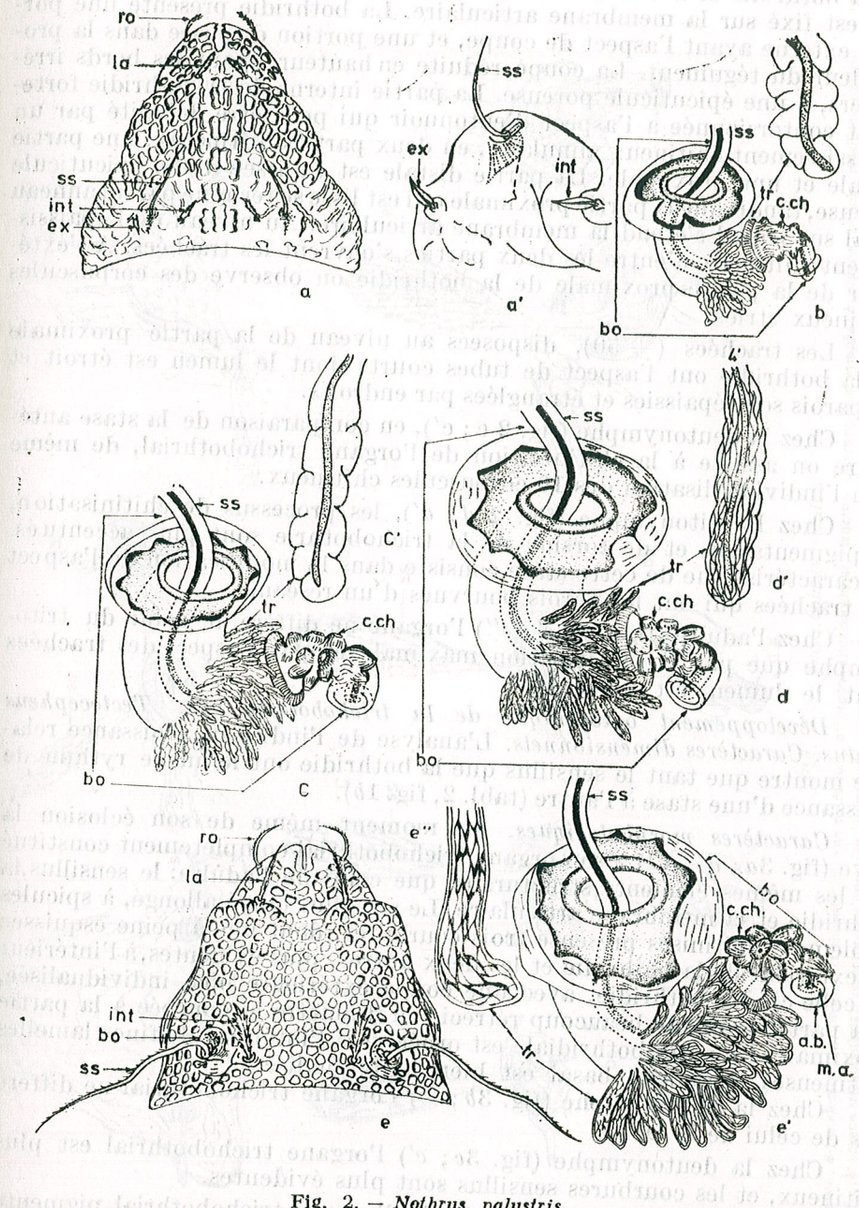

Fig. 2. — *Nothrus palustris*.

Larve: *a*, prodorsum; *a'*, sensillus; *Protonymphe*: *b*, organe trichobothrial; *b'* trachée; *Deutonymphe*: *c*, organe trichobothrial; *c'*, trachée; *Tritonymphe*: *d*, organe trichobothrial; *d'*, trachée; *Adulte*: *e*, prodorsum; *e'*, organe trichobothrial; *e''*, trachée. ro, poil rostral; la, poil lamelaire; int, poil interlamelaire; ex, poil exobothrial; ss, sensillus; bothridie; c.ch, corpuscule chitineux, ab anneau basal; ma membrane articulaire; tr, trachée.

de la bothridie et deux à l'intérieur de celle-ci. Le bout proximal du sensillus est fixé sur la membrane articulaire. La bothridie présente une portion externe ayant l'aspect de coupe, et une portion disposée dans la profondeur du tégument. La coupe, réduite en hauteur, ayant les bords irréguliers, a une épicuticule poreuse. La partie interne de la bothridie fortement contorsionnée a l'aspect d'entonnoir qui peut être délimité par un épaississement chitineux annulaire, en deux parties distinctes : une partie distale et une proximale. La partie distale est tapissée d'une épicuticule poreuse, tandis que la partie proximale qui est lisse se termine par un anneau basal sur lequel s'étend la membrane articulaire. Au niveau de l'épaississement chitineux d'entre les deux parties s'ouvrent les trachées. A l'extérieur de la partie proximale de la bothridie on observe des corpuscules chitineux striés.

Les trachées ($\pm$ 50), disposeés au niveau de la partie proximale de la bothridie ont l'aspect de tubes courts dont le lumen est étroit et les parois sont épaissies et étranglées par endroits.

Chez la deutonymphe (fig. 2 c ; c′), en comparaison de la stase antérieure on assiste à la chitinisation de l'organe trichobothrial, de même qu'à l'individualisation des 10 corpuscules chitineux.

Chez la tritonymphe (fig. 2 *d* ; *d′*), les processus de chitinisation, de pigmentation et de torsion de la trichobothrie sont plus accentués. La caractéristique de cette stase consiste dans la modification de l'aspect des trachées qui ont les parois pourvues d'un réseau.

Chez l'adulte (fig. 2*e* ; *e′* ; *e″*) l'organe ne diffère de celui du tritonymphe que par sa chitinisation maximale et par l'aspect des trachées dont le lumen est élargi.

Développement ontogénique de la trichobothrie chez Tectocepheus velatus. Caractères dimensionnels. L'analyse de l'indice de croissance relative montre que tant le sensillus que la bothridie ont le même rythme de croissance d'une stase à l'autre (tabl. 2, fig. 1*b*).

Caractères morphologiques. Au moment même de son éclosion la larve (fig. 3*a* ; *a′* ; *a″*) a son organe trichobothrial complètement constitué par les mêmes éléments structuraux que celui de l'adulte: le sensillus, la bothridie et la membrane articulaire. Le sensillus clavé-allongé, à spicules faiblement chitinisés présente trois courbures, dont une à peine esquissée à l'extérieur de la bothridie et les deux autres, plus évidentes, à l'intérieur de celle-ci. La bothridie, avec une coupe extérieure bien individualisée, a la partie profonde beaucoup rétrécie en diamètre et courbée à la partie proximale. La paroi bothridiale est ornée vers l'intérieur de fines lamelles chitineuses. L'anneau basal est bien individualisé.

Chez la protonymphe (fig. 3*b* ; *b′*) l'organe trichobothrial ne diffère pas de celui de la larve.

Chez la deutonymphe (fig. 3*c* ; *c′*) l'organe trichobothrial est plus chitineux, et les courbures sensillus sont plus évidentes.

Chez la tritonymphe (fig. 3*d* ; *d′*) l'organe trichobothrial pigmenté et chitinisé a la partie profonde de la bothridie uniformément épaissie.

Chez l'adulte (fig, 3*e* ; *e′* ; *e″*) l'organe trichobothrial atteint le maximum de chitinisation et de torsion.

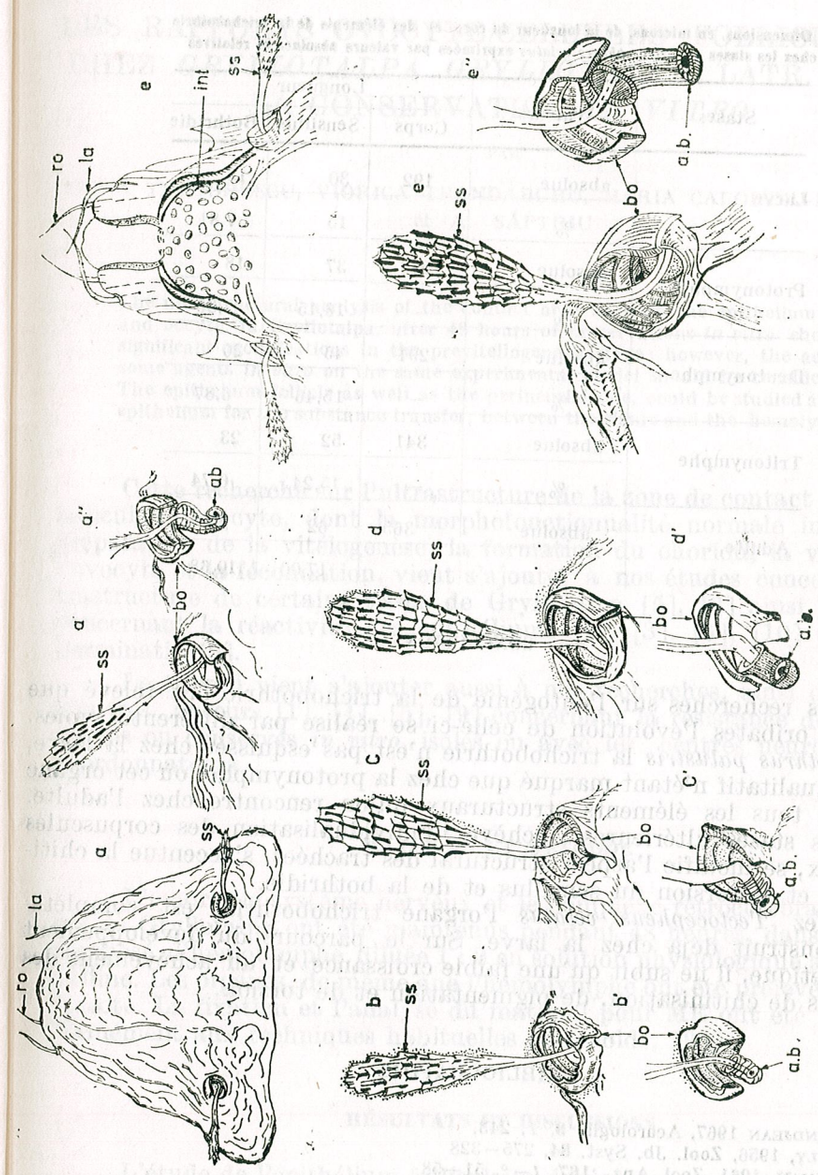

Fig. 3. — *Tectocepheus velatus*.

Larve : *a*- prodorsum ; *a*, organe trichobothrial, vue extérieure, *a″* organe trichobothrial, structure interne ; *Protonymphe* ; *b* organe trichobothrial, vue extérieure ; *b′*, organe trichobothrial, structure interne ; *Deutonymphe* : *c*, organe trichobothrial, vue extérieure ; *c′*, organe trichobothrial, structure interne ; *Tritonymphe* : *d*, organe trichobothrial, vue extérieure ; *d″*, organe trichobothrial vue extérieure ; *e″*, organe trichobothrial, structure interne. *Adulte* : *e*, prodorsum ; *e′*, organe trichobothrial vue extérieure ; *e″*, organe trichobothrial, structure interne, ro, poil rostral ; la, poil lamellaire ; int, poil intermédiaire ; ss, sensillus ; bo, bothridie ; anneau basal ; ma, membrane articulaire.

Tableau 2

Dimensions, en microns, de la longueur du corps et des éléments de la trichobothrie chez les stases de *Tectocepheurs velatus* exprimées par valeurs absolues et relatives

Stases	Valeurs	Longueur		
		Corps	Sensillus	Bothridie
Larve	absolue	192	30	15
	%	—	15	7,81
Protonymphe	absolue	229	37	18
	%	—	16,15	7,86
Deutonymphe	absolue	291	45	20
	%	—	15,46	6,87
Tritonymphe	absolue	341	52	23
	%	—	15,24	6,74
Adulte	absolue	365	62	39
	%	—	17,00	10,68

CONCLUSIONS

Nos recherches sur l'ontogénie de la trichobothrie ont relevé que chez les oribates l'évolution de celle-ci se réalise par différentes voies. Chez *Nothrus palustris* la trichobothrie n'est pas esquissée chez la larve, le saut qualitatif n'étant marqué que chez la protonymphe, où cet organe présente tous les éléments structuraux qu'on rencontre chez l'adulte. Dans les stases ultérieures s'achève l'individualisation des corpuscules chitineux, se modifie l'aspect structural des trachées, s'accentue la chitinisation et la torsion du sensillus et de la bothridie.

Chez *Tectocepheus velatus* l'organe trichobothrial est complètement construit déjà chez la larve. Sur le parcours du développement ontogénetique, il ne subit qu'une faible croissance et un achèvement des processus de chitinisation, de pigmentation et de torsion.

BIBLIOGRAPHIE

1. F. Grandjean 1967, Acarologia, **9**, *1*, 243.
2. F. Pauly, 1956, Zool. Jb. Syst. **84**, 275—328
3. K. Tarman, 1961, Zool. Anz., **167**, *1—2*, 51—58.

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LES RAPPORTS OVOCYTE/CELLULES FOLLICULAIRES CHEZ *GRYLLOTALPA GRYLLOTALPA* LATR. APRÈS LA CONSERVATION *IN VITRO*

PAR

MARIA TEODORESCU, VIORICA TRANDABURU, MARIA CALOIANU-IORDĂCHEL et A. SĂFTOIU

The ultrastructural analysis of the contact area between the epithelium follicle and oocyte, in Gryllotalpa, after 48 hours of preservations *in vitro* shows less significant modifications in the previtellogenesis stage; however, the action of some agents *in vitro* on the same experimental model should be considered. The epithelium follicle as well as the perineurial one, could be studied as a bar epithelium for the substance transfer, between the tissue and the hemolymph.

Cette recherche sur l'ultrastructure de la zone de contact épithélium folliculaire/ovocyte, dont la morphofonctionnalité normale influence la préparation de la vitélogenèse, la formation du chorion, la viabilité de l'ovocyte et la fécondation, vient s'ajouter à nos études concernant l'ultrastructure de certains tissus de Gryllotalpa [5], [8] ainsi qu'à celles concernant la réactivité des épithéliums [2], [3], [6], [10] et du tissu germinatif [9].

Le travail vient s'ajouter aussi à nos recherches, ainsi qu'à celles d'autres auteurs [1], [4], [5], [9] concernant la résistance des organels greffés ou conservés *in vitro*, isolés ou avec les centres neuroendocrines coordonnateurs.

MATÉRIEL ET MÉTHODE

L'ovaire, le système nerveux et le complexe rétrocérébral des adultes de Gryllotalpa ont été maintenus pendant 48 heures, dans un milieu composé d'hémolymphe diluée 1 : 2 en solution physiologique avec Ampicylline. Les organes, de même que l'hémolymphe ont été prélevés du même insecte. La fixation et l'analyse du matériel pour ME ont été faites conformément aux techniques habituelles (Reynolds, 1962).

RÉSULTATS ET DISCUSSIONS

L'étude de l'épithélium folliculaire de l'ovocyte prévitélogène, effectuée par nous antérieurement [9] a mis en évidence des cellules variables en ce qui concerne la forme et le stade fonctionnel : cellules hautes, cubi-

ques, aplaties, ces dernières manifestant des aspects de dégradation. Dans le cas de certains follicules, nous avons signalé des aspects d'altération dans l'ovocyte, ainsi que dans l'épithelium folliculaire, mais seulement après 48 heures de culture *in vitro*.

En examinant dans le présent travail, l'ultrastructure de la zone de frontière entre l'ovocyte et les cellules folliculaires, nous avons constaté pourtant que certains follicules résistent pendant 48 heures *in vitro*, de manière que les rapports morphofonctionnels entre les cellules folliculaires et l'ovocyte restent encore actifs.

L'épithélium folliculaire de tels follicules prévitélogènes reste unistratifié, présentant les mêmes types de cellules que celles présentées plus haut. Les cellules nous apparaissent « denses » et « claires » (fig. 1) jonctionnées entre elles par des expansions crampon, zonules occludens et des mosomes. La face extérieure des cellules, vers le chorion, forme parfois de rares excrescences, courtes et inégales. La face intérieure, vers l'ovocyte, présente de nombreux microvilles, souvent très longs, quelquefois aglomérés en houppes.

Fréquemment, les microvilles plus longs ne sont pas rectilignes et parallèles en direction aux prolongements de l'ovocyte.

L'interpénétration de ces deux types de prolongements est tellement intime, que l'espace intercellulaire reste très étroit. Les prolongements de l'ovocyte (fig. 2 a_1, a_2, a_3, a_4) à leur endroit d'émergence sont volumineux, de forme variable souvent ramifiés ; ils sont séparés par des rainures étroites, courtes ou profondes. Etant donné la direction perpendiculaire des microvilles des cellules folliculaires, souvent ils apparaissent coupés à travers et groupés dans les espaces compris entre les prolongements de l'ovocyte (fig. 2). Le cytoplasme des cellules folliculaires « denses » et « claires » contient du REG, ainsi que des polysomes isolés, des corps plurivésiculaires clairsemés, un complexe Golgi peu développé, de petites mitochondries sphériques, de rares microtubules, des corps miéloïdes et des corps denses très rares. Dans les cellules « denses » les citernes REG présentent un lumen très étroit, souvent difficilement visible même aux augmentations fortes.

Par contre, dans les cellules « claires », le lumen s'élargit et les citernes confluentes donnent au REG l'aspect lacuneux. Les citernes REG élargies ne perdent pas la liaison avec la citerne périnucléaire. Elles contiennent un produit intralacuneux, qui semble être transféré par le système des lacunes à la périphérie des cellules, dans l'espace intracellulaire d'où il arrive dans l'espace périovocytaire. La présence des lacunes à la face extérieure des cellules folliculaires, ainsi qu'à la base et dans la profondeur des microvilles, atteste que ce produit utilise aussi cette voie directe d'élimination (fig. 3). Le processus de formation et d'élimination de la sécrétion des cellules folliculaires sollicite, ainsi que nous l'avons vu, exclusivement le REG, en évitant la voie habituelle, mais plus détournée : REG, zone Golgi, plasmalème. Ce fait montre que le processus découle rapidement, même sans être nécessaire une phase de stockage du produit élaboré. REG est sollicité en même temps et, probablement avec la même rapidité, dans le processus de redressement des citernes REG, processus dans lequel la citerne périnucléaire intervient évidemment (fig. 4).

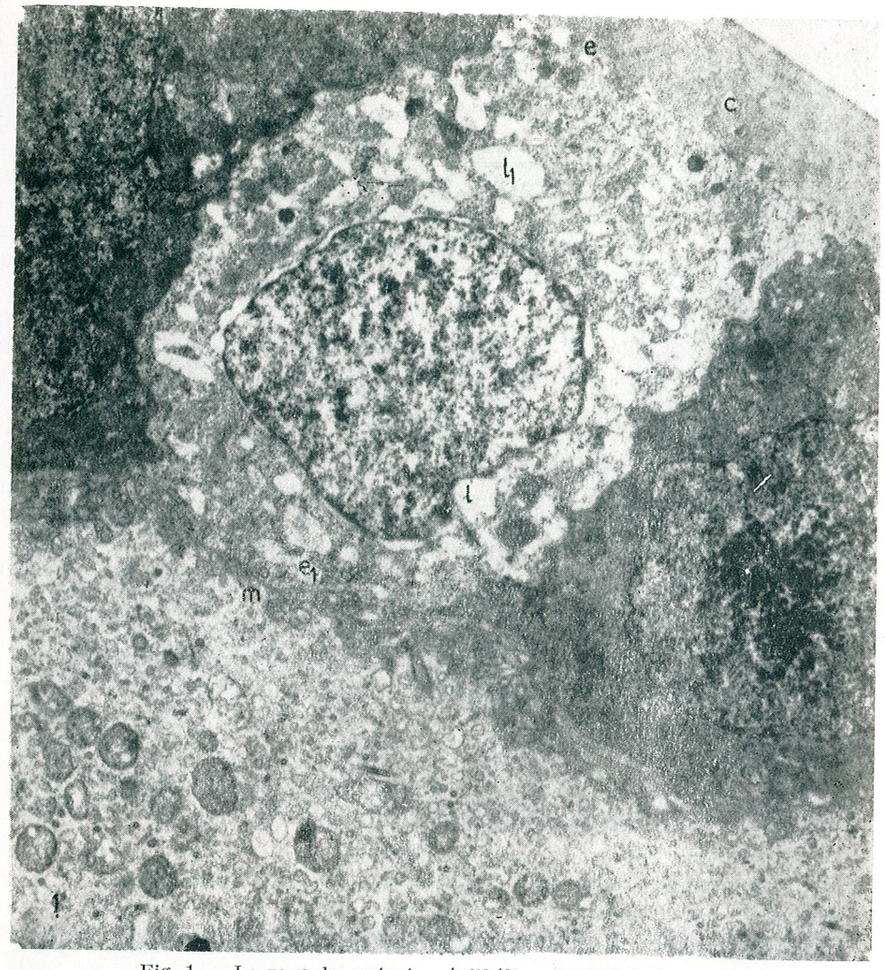

Fig. 1. — La zone de contact — épithélium folliculaire/ovocyte ;
m — microvilles dans l'espace périovocytaire ; 1 — lacunes de la citerne périnucléaire ; 11 — les lacunes citernales du REG ; *e* — l'exocytose du produit vers le chorion (c) ; e_2 — l'exocytose du produit vers l'ovocyte × 2140.

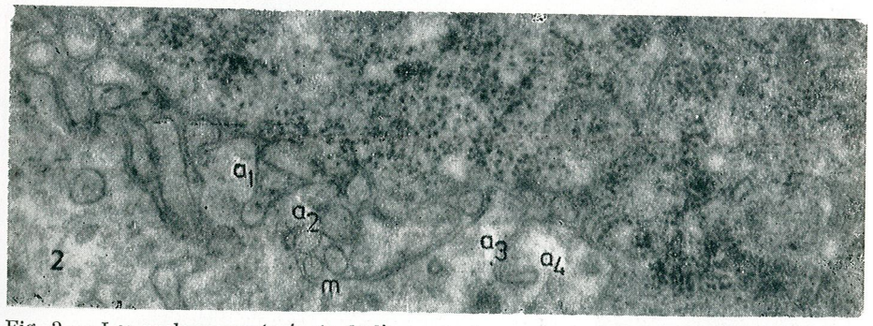

Fig. 2. — Les prolongements épais de l'ovocyte (a_1, a_2, a_3, a_4). Les microvilles (*m*) d'une cellule « dense » au voisinage des prolongements de l'ovocyte × 9800.

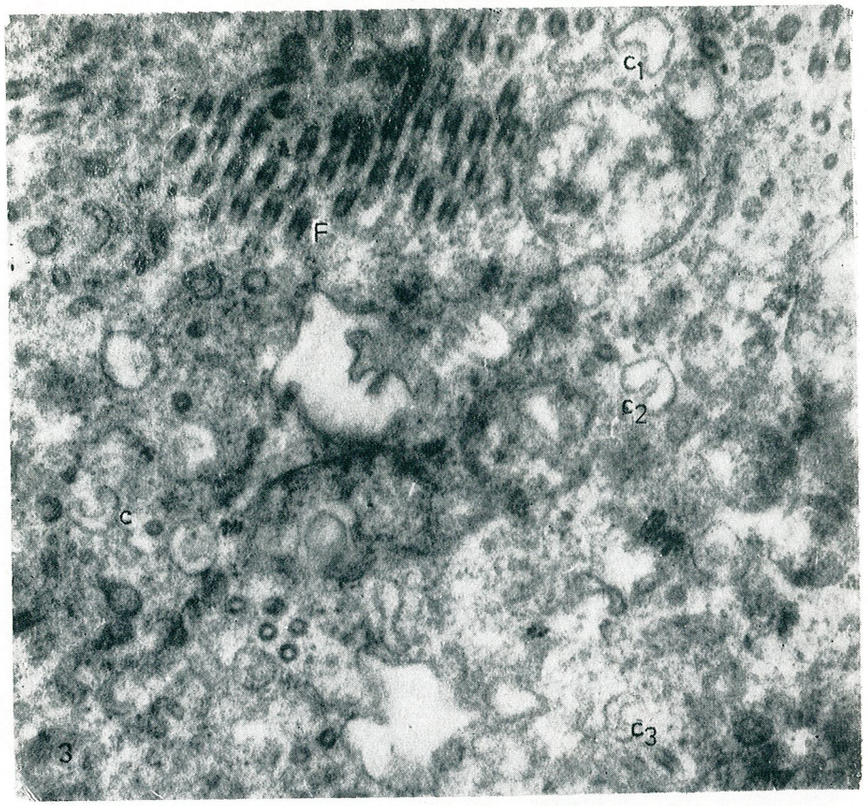

Fig. 3. — Organites cytoplasmiques dans l'ovocyte.
F — faisceau de microtubules ; citerne REN en forme de coupe ($c_1\ c_2\ c_3$) × 6450.

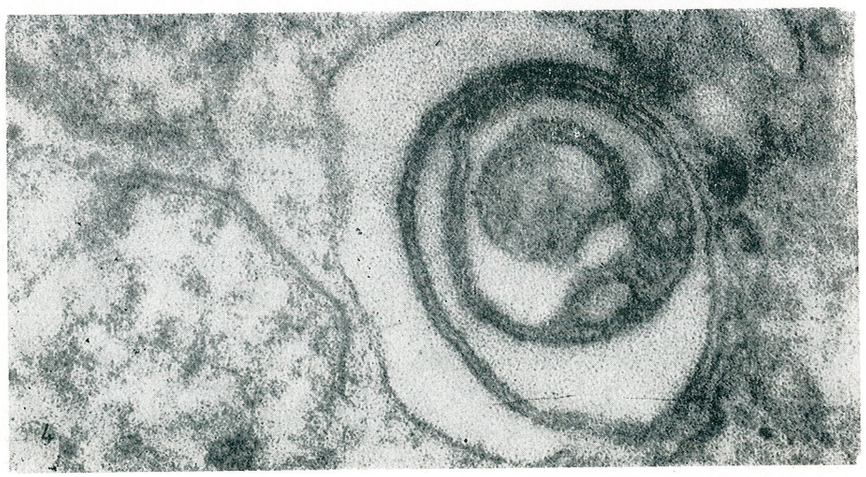

Fig. 4. — Figure miélinique et une mitochondrie altérée dans le cytoplasme de l'ovocyte × 14 000.

Dans l'épithélium folliculaire, la plupart des cellules paraissent actives, en appréciant l'aspect du noyau. Les noyaux, sont en général, faiblement chromatiques avec des nucléoles bien développés ayant la nucléolonème polymorphe. La forme du noyau diffère ; dans le même type de cellule, « claire », ou « dense », le noyau peut être sphérique, ovoïde avec une surface lisse, avec des incisures étroites ou des excavations larges. Le noyau approche soit de la face externe, soit de celle interne, isolé de la plasmalème seulement par une très fine pellicule de cytoplasme. C'est une autre preuve de l'activité des cellules folliculaires.

A la suite du fait que le pôle interne des cellules folliculaires est bien proéminent vers l'ovocyte, la surface de ce dernier ne reste plus lisse mais avec des proéminences enfoncées comme des papilles dans la direction des zones de jonction entre les cellules folliculaires.

L'ovocyte à 48 heures de conservation *in vitro* contient à la périphérie un cytoplasme plus pauvre en organites que dans le reste. Il contient des microtubules et de nombreuses vésicules de pinocytose avec un contenu de densité variable. Certaines organites ovocytaires manifestent des aspects d'épuisement. De la sorte, les mitochondries, d'habitude petites, sphériques ou allongées, paraissent quelquefois déformées ou détruites en partie. Même aux mitochondries dont l'aspect est normal, la membrane extérieure est plus épaisse et plus électrono-dense que celle intérieure. Le compartiment externe est clair et en général, de même grandeur.

La structuralité différente de la matrice confère un aspect variable au compartiment intérieur : claire, fin granulé, avec des densifications locales plus ou moins étendues. Les cristes de la membrane intérieure sont très rares, avec un trajet plus ou moins ordonné dans la direction de la paroi voisine avec laquelle rarement elles fusionnent. Souvent, les cristes manquent.

Les microtubules sont isolés, groupés à raison de 2 ou 3, ou en faisceau, composés de 30 — 40 microtubules. L'arrangement des microtubules, dans les sections transversales, rappelle la disposition hexagonale des miofilaments de la fibre musculaire striée. Le diamètre et la position équidistante des microtubules se conservent, sans égard au territoire cytoplasmique analysé. Il est possible que la rétraction ou un processus de dépolymérisation-répolymérisation des microtubules facilitent le déplacement des organites vers les zones plus actives du cytoplasme de l'ovocyte.

Dans la zone périphérique, mais aussi dans le reste de l'ovocyte, on rencontre de nombreuses citernes REN sphériques, et surtout en forme de coupe, ayant dans leur excavation un cytoplasme plus dense que dans le reste. Les extrémités de la citerne approchent et fusionnent, ou se collent, la paroi de contact devenant commune.

Ces recherches prouvent que les follicules prévitélogènes restent viables pendant 48 heures *in vitro* et c'est dans cet intervalle qu'on peut actionner expérimentalement sur eux. Cette viabilité est entretenue par l'influence exercée dans le milieu de culture par les centres neuroendocrines. L'analyse de la zone épithélium folliculaire/ovocyte au niveau ultrastructural prouve que la réactivité est maintenue à ce niveau, ce qui

confirme et complète les recherches antérieures, menées par nous ou par d'autres auteurs [2], [3], [6], [7], [9], [10].

Les altérations que nous avons signalées au niveau mitochondrial peuvent être attribuées à la sollicitation physiologique, mais aussi à la condition expérimentale.

Les recherches suivantes sur le même modèle expérimental devront tenir compte de ces modifications dans l'appréciation des résultats.

BIBLIOGRAPHIE

1. Didier R., Bull. Soc. Zool. Fr., 1974, **99**, 93.
2. Metcalf R. L., Fukuto T. R., March R. B., J. Econ. Ent., 1959, **52**, 44.
3. Renberg S., Z. Zellf. Anat., 1966, **72**, 379.
4. Riviere J. L., Bull. Soc. Zool. Fr., 1974, **99**, 121.
5. Teodorescu M., Caloianu-Iordăchel M., Rev. Roum. Biol. — Zoologie, 1973, **18**, 305.
6. Teodorescu M., Trandaburu V., Văcaru A., Rev. Roum. Biol. 1974, **19**, 101.
7. Teodorescu M., Trandaburu V., Rev. Roum. Biol. — Biol. Anim. 1976, **21**, 61.
8. Teodorescu M., Caloianu-Iordăchel M., Rev. Roum. Biol. — Biol. Anim., 1976, **21**, 123.
9. Teodorescu M., Trandaburu V., Caloianu-Iordăchel M., Rev. Roum. Biol. — Biol. Anim., 1978, **23**, 21.
10. Weygoldt P., Zool. Anz. 1971, **166**, 69.

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CYTOCHEMICAL LOCALIZATION AND ELECTROPHORETICAL CHARACTERIZATION OF ACID PHOSPHATASE IN FISH OVARY

BY

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In the cortical region of fish oocytes (*Carassius auratus gibelio* and *Cyprinus carpio*), cortical granules with high acid phosphatase activity were histochemically and cytochemically identified. Weak activity was found in the Golgi system and few lysosome-like structures of young oocytes. Electrophoretical characterization of acid phosphatase from yolk platelets and cortical granules fraction, solubilized in sodium acetate buffer and non-ionic detergent (triton X-100) revealed a peculiar electrophoretic pattern, which is dependent on substrate and pH. Two non-specific substrates (β glycerophosphate and naphthylphosphate) and three natural substrates (two caseins and phosvitin) at varying pH (3.0 and 5.0) were employed. The data suggest the possible function of acid phosphatase as phosphoprotein phosphatase.

Acid phosphatase activity (orthophosphoric-monoester phosphohydrolase, acid optimum, EC 3.1.3.1) as a marker enzyme for lysosomes has been questioned, sice the enzyme was localized cytochemically at various extralysosomal sites, in different tissues [1], [3], [10], [11]. Our previous study showed the presence of acid phosphatase in young and developed oocytes as intensely stained granules, which participate in the formation of yolk platelets [14].

In order to gain additional information, we tried in this study to approach the problem of distribution of acid phosphatase in fish ovaries by an ultrastructural study. Moreover, we compared electrophoretically the enzyme activities from yolk platelets fraction, revealed after incubation in the presence of several substrates at two different pH. The degree of specificity of the non-specific activities of acid phosphatase were compared electrophoretically with some natural substrates (casein and phosvitin). The results provide evidence for a probable regulatory function of acid phosphatase as phosphoprotein phosphatase in fish ovary.

MATERIAL AND METHODS

Our experiments were carried out with two fish species, namely crucian carp (*Carassius auratus gibelio*) and common carp (*Cyprinus carpio*), supplied by the Nucet piscicultural research station.

Histochemistry. The ovary was removed, minced in small fragments and washed in saline solution of NaCl 0.6%. The tissues were fixed in 2%

glutaraldehyde in cacodylate buffer solution 0.05 M (pH 7.4), for one hour at 4°C. The pieces were washed several times with cacodylate buffer solution. Frozen sections (10μ) were prepared and incubated in specific media, by using the Gomori technique and the coupling method with azo-stains [5].

Electron-microscopy. Small pieces of ovary were fixed in 2% glutaraldehyde prepared in cacodylate buffer solution 0.05 M (pH 7.4) for one hour and then washed four times successively with cold cacodylate buffer solution. For cytochemical demonstration of acid phosphatase, we used an incubation medium consisting of: sodium acetate buffer solution 0.2 M (pH 5.0), lead nitrate 3mM, beta glycerophosphate or naphthylphosphate 6 mM. Incubation was carried out for 2—4 h, at 30°C. After incubation, the pieces were post-fixed in 1% OsO_4, dehydrated in cold ethanol series and embedded in Epon. Ultrathin sections were examined either unstained or stained for a short time with uranyl acetate and lead citrate.

Electrophoresis. Fresh ovaries were minced in small fragments in saline solution of NaCl 0.6%, homogenized in a Potter homogenizer in distilled water, at a dilution of 1 : 10 (g/v). The homogenate was filtered through one layer of nylon cloth, and then by differential centrifugation, the yolk platelets and cortical granules fraction were separated (8 000 g for 15 min). This fraction was extracted with sodium acetate buffer 0.2 M (pH 5.0) or tris-HCl buffer 0.1M (pH 7.0) and, in parallel, in the same solutions with detergent triton X-100 (1%).

The disc-electrophoresis method on polyacrylamide gel was used according to Davis' technique [4]. For the identification of acid phosphatase on gels, we used several substrates in the incubation medium (similarly to the cytochemical technique), at two different pH (3.0 and 5.0). The following substrates were employed: beta glycerophosphate, naphthylphosphate, casein Hammersten, casein prepared by the Cantacuzino Institute and phosvitin prepared in our laboratory.

The protein on gels was revealed with amido-black solution 1% and Comassie blue solution 1%, prepared in acetic acid 3%.

Phosvitin purification from avian egg yolk was effected by the procedure designed by Burley and Cook [2] and Wallace *et al.* [13].

RESULTS

1. *Histochemical localization of acid phosphatase.* Histochemical localization of acid phosphatase was followed up in the ovaries of two fish species, in the presence of β glycerophosphate used as substrate. The enzymatic reaction product appears under the form of granules spread in the cytoplasm among cortical vacuoles, particularly under oolema. In this portion of the cell, numerous granules gather intensely stained (Pl. I, C and D). In the oocytes of both fish species by means of two different methods (diazonium salts and lead nitrate), the acid phosphatase is histochemically similarly localized. These cortical granules, with intense acid phosphatase reaction, contribute to the formation of yolk platelets.

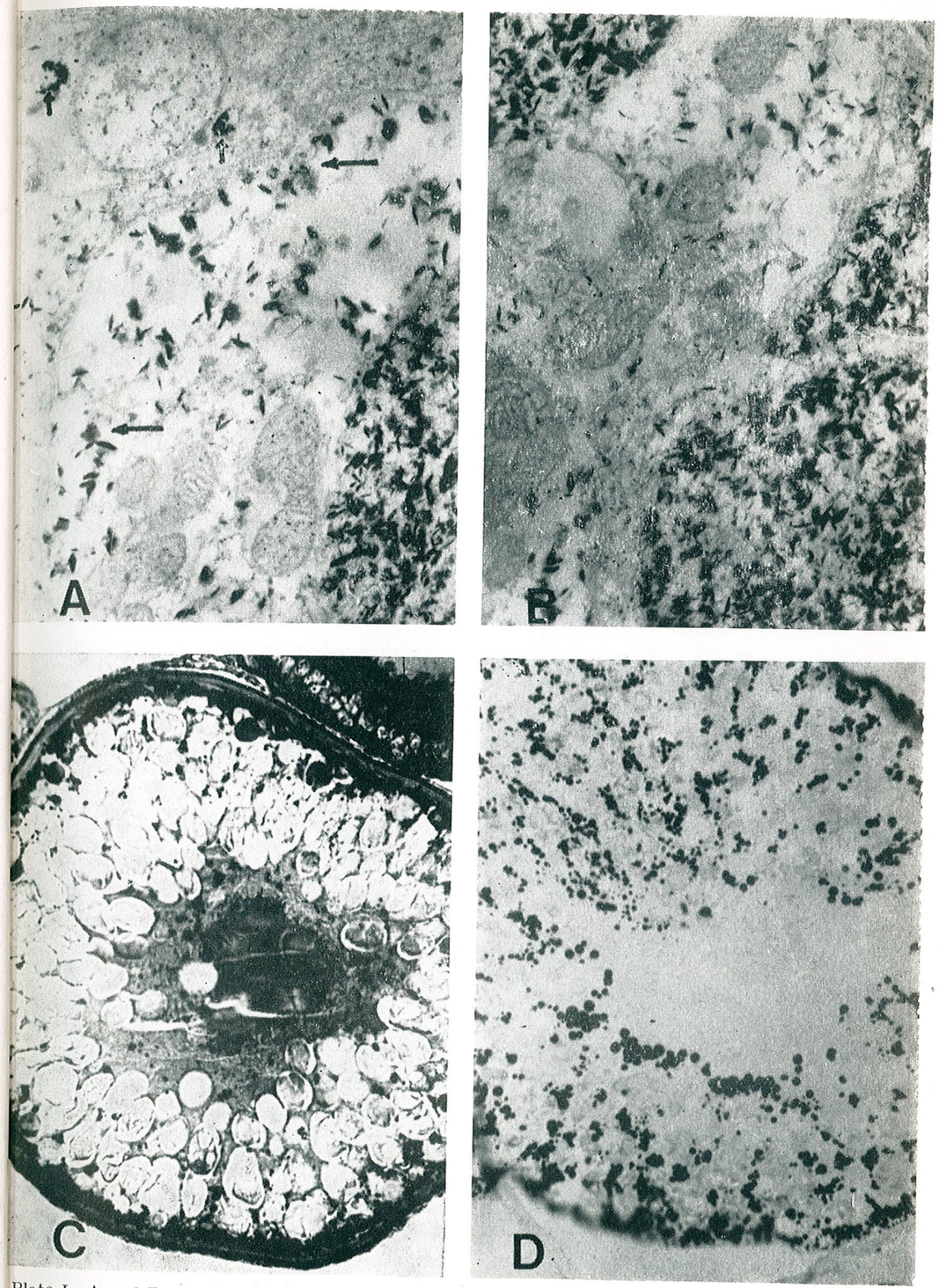

Plate I: A and B. — Ultrastructural localization of acid phosphatase in fish ovary *(Cyprinus carpio)*. Cortical granules with positive reaction are observed (× 4500).
C and D: Histochemical localization of acid phosphatase in fish ovary (*Cyprinus carpio*); azocoupling method (C) and Gomori method (D).

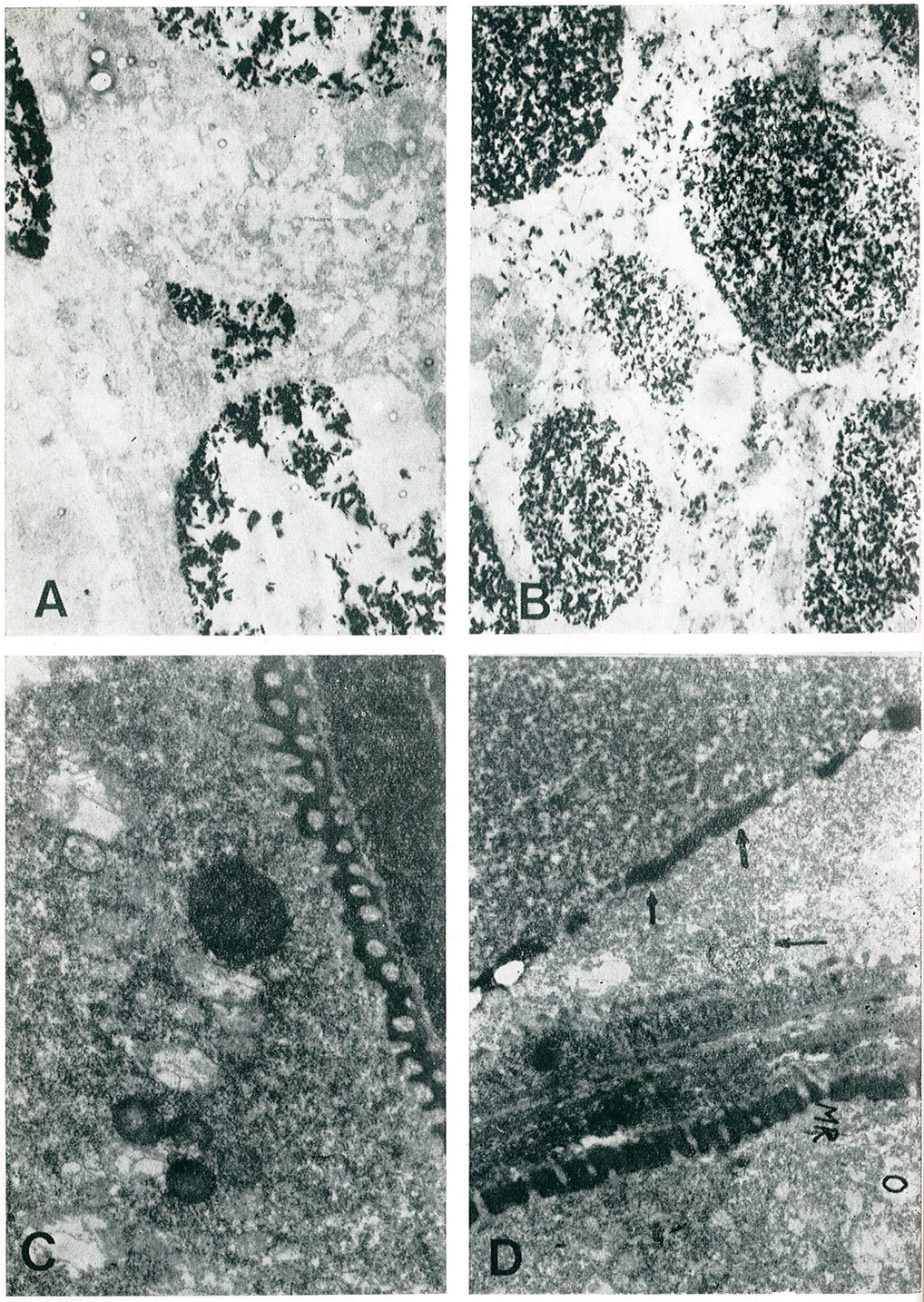

Plate II : A and B. — Ultrastructural localization of acid phosphatase in fish ovary (*Carassius auratu gibelio*). Cortical granules with high precipitate of lead phosphate are seen (× 450
C and D — Young fish oocytes from crucian carp ; note the presence of the reaction product the level of the plasma membrane of the epithelial cells and in some lysosome structures (× 4500

2. *Ultrastructural localization of acid phosphatase.* The ultrastructural study focused our attention on the identification of the cortical granules in the peripheral area of oocytes. In young crucian carp oocytes the acid phosphatase showed a weak cytochemical reaction, at the level of lysosome-like structures (Pl. II, C and D). The presence of acid phosphatase was not identified in the radiate membrane or in the follicular cells. A similar distribution of the acid phosphatase was also observed on the common carp oocyte. Moreover, the enzyme was present in the membranes of epithelial cells of blood vessels, which are in close morphological and functional correlation with the follicular cells and the developing oocytes (Pl. II, D).

In the cortical portion of well-developed oocytes, the enzyme was identified cytochemically at the level of some granules, named cortical granules. These latter showed an intense positive reaction and were found in both common and crucian oocytes (Pl. I and II A and B). Aside from the preferential localization in these cortical granules, the enzyme was also found—but with poor activity — in the cisternal vesicles of the Golgi apparatus (Pl. I, A) as well as at the level of smaller structures, which we considered as lysosomes (Pl. I,A).

3. *Electrophoretic study.* In a first stage, we used several solubilizing media (distilled water, acetate buffer 0.2 M at two different pH, tris-HCl buffer 0.1 M at pH 7.0), used as such or combined with triton X-100. The use of this detergent was suggested by a previous study by which it was found that acid and alkaline phosphatase did not significantly modify their activity in the presence of triton X-100 [6]. Moreover, the enzymes maintained their biological activities in the presence of high concentrations of detergent [7], [12]. The best solubilization was achieved in acetate buffer 0.2M pH 5.0 with triton X-100 in 1% concentration. The electrophoregram of platelets fraction proteins showed 22 proteic bands in the presence of detergent and only 17 in its absence (Fig. 1).

The activity of solubilized acid phosphatase from the yolk platelets fraction has been electrophoretically studied in the presence of two synthetic substrates at pH 3.0 and 5.0 (Fig. 2). As can be seen, the identification of the enzyme at a low pH (Fig. 2,A) uncovers a small number of molecular fractions with enzymatic activity. By incubation of gels at pH 5.0, several fractions with enzymatic activity were identified (Fig. 2, B). Thus, in the presence of β glycerophosphate 8 enzymatic fractions appeared on the gel. Six fractions were identified with naphthylphosphate as substrate, whose position in the gel was similar to the glycerophosphate phosphatasic bands.

The activity of acid phosphatase was electrophoretically studied also in the presence of some natural substrates, under experimental conditions identical with those for synthetic substrates. The enzymatic activity has been tested, in parallel, also in the postmitochondrial fraction. In the presence of the 3 natural substrates (pH 5.0), the electrophoregrams appear similar, irrespective of the natural substrate used (Fig. 3). At pH 3.0 poor enzymatic activities were obtained, both in postmitochondrial fraction and platelets fraction (Fig. 4). At this pH value, in the presence of phosvitin, no enzymatic activity was obtained in either fraction investigated.

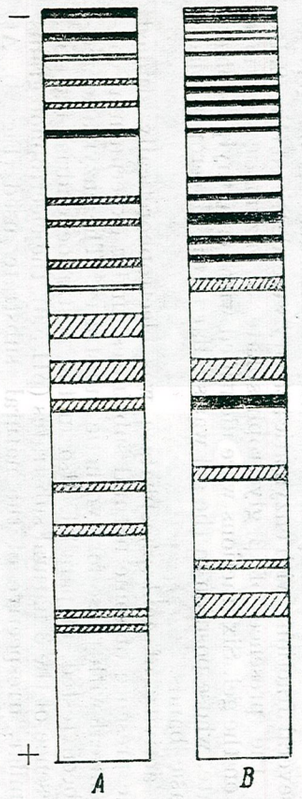

Fig. 1. — Electrophoretical patterns of proteins from platelets fraction of fish ovary, extracted in sodium acetate buffer 0.2 M, pH 5.0 (A) and

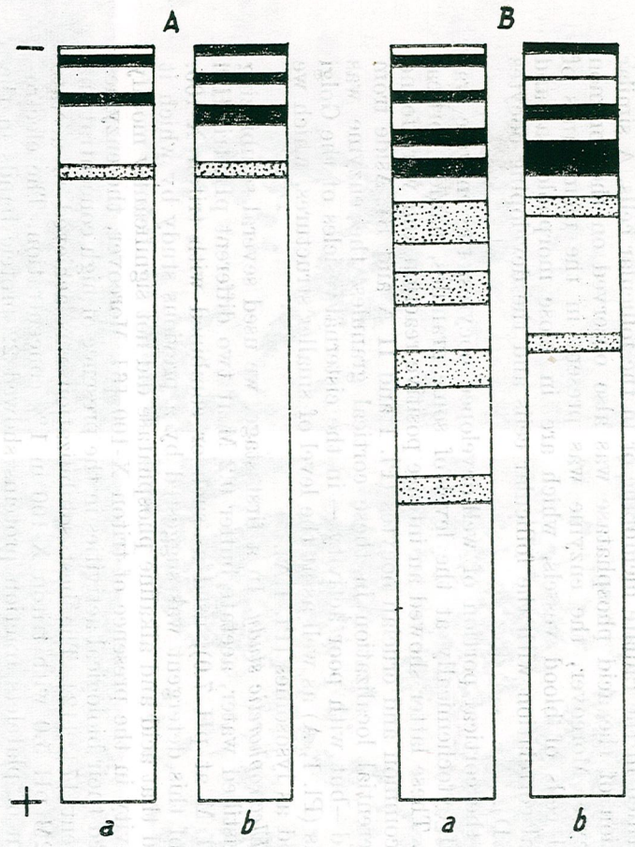

Fig. 2. — Electrophoretical patterns of acid phosphatase from platelets fraction of fish ovary, by incubation in the β glycerophosphate (a) or α naphthylphosphate (b); pH 3.0 (A) and pH 5.0 (B).

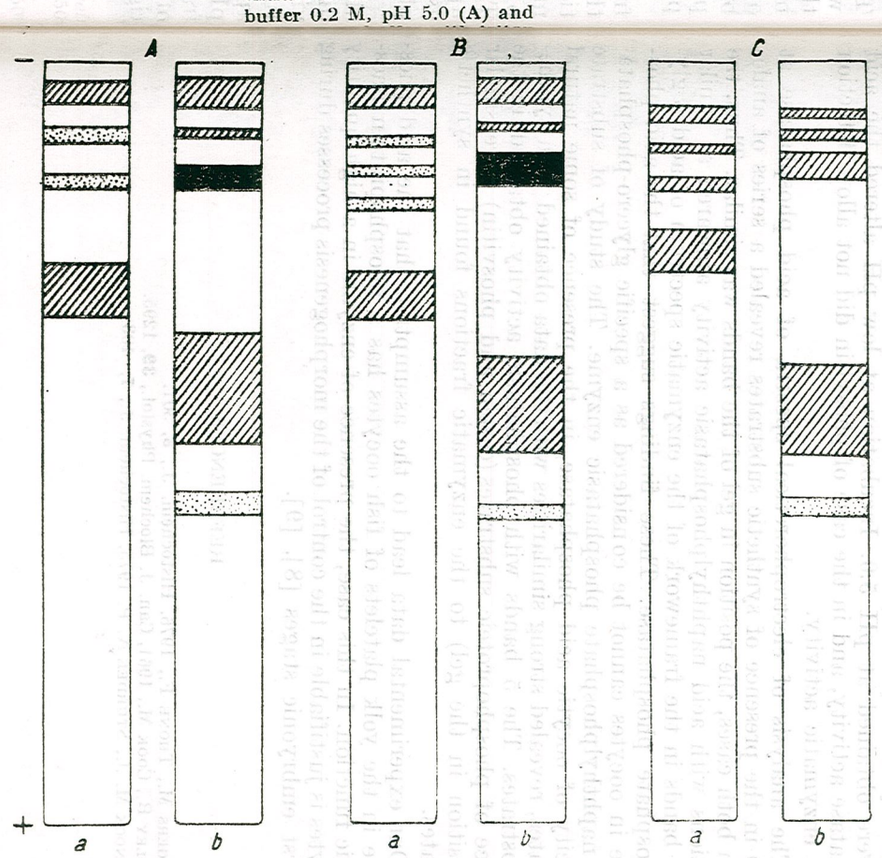

Fig. 3. — Electrophoretical patterns of acid phosphatase from soluble postmitochondrial fraction (a) and platelets fraction (b) of fish ovary, by incubation of gels at pH 5.0 in the presence of natural substrates : A) case in Hammersten ; B) case in Cantacuzino Inst. ; C) phosvitin.

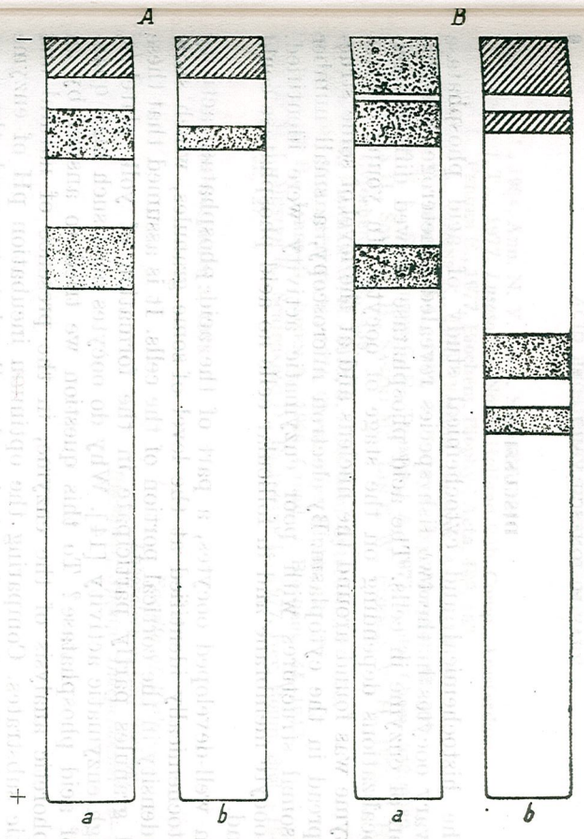

Fig. 4. — Electrophoretical patterns of acid phosphatase from soluble postmitochondrial fraction (a) and platelets fraction (b) of fish ovary, by incubation of gels at pH 3.0 ; A) case in Hammersten ; B) case in Cantacuzino Inst.

DISCUSSION

The histochemical and cytochemical study of acid phosphatase from ovary oocytes in the two fish species revealed a heterogeneous distribution of enzyme in cells. The acid phosphatase showed different cellular localizations depending on the stage of oocytes. In young oocytes the enzyme was found around the nucleus and at the level of some structures spread in the cytoplasm. By electron microscopy, a small number of lysosomal structures with poor enzymatic activity were identified. In the oocyte membrane and in follicular cells no acid phosphatase could be found.

In well-developed oocytes, a part of the acid phosphatase activity was cytochemically identified at the level of some granules, which show a high density in the cortical portion of the cells. It is assumed that these cortical granules partly participate in the formation of yolk platelets, with high enzymatic activity [14]. Why do oocytes need such high quantities of acid phosphatase? To this question we tried to answer by an electrophoretic analysis of the enzyme, in the presence of natural and synthetic substrates. Comparing the optimum incubation pH of enzyme on polyacrylamide gel, we found that for all substrates used, the most obvious enzymatic spectra (as to the number of bands and staining intensity) were obtained at pH 5.0. Incubation at low pH altered the acid phosphatase activity, and in the case of phosvitin did not allow detection of any enzymatic activity.

The analysis of electrophoretical spectra of acid phosphatase at pH 5.0 in the presence of synthetic substrates revealed a series of analogies. In both cases, the position in gel of the bands was similar. Thus, the 6 fractions with acid naphthylphosphatasic activity appeared as similar with 6 bands in the framework of the enzymatic spectrum of acid β glycerophosphate phosphatase. These findings suggest that the acid phosphatase in oocytes cannot be considered as a specific glycero-phosphatasic of naphthylphosphate phosphatasic enzyme. The study of substrate specificity of oocytic acid phosphatase, in the presence of some natural substrates, revealed strong similarities with the data obtained with synthetic substrates. The 5 bands with phosphatasic activity obtained in the presence of phosphoproteic substrates (casein and phosvitin) were similar (as position in the gel) to the enzymatic fractions found in synthetic substrates.

Our experimental data lead to the assumption that the acid phosphatase in the yolk platelets of fish oocytes has a phosphoprotein phosphatasic function. In this case, the presence of enzyme in a high quantity in oocytes is justifiable in the control of the morphogenesis processes during the first embryonic stages [8], [9].

REFERENCES

1. Borgers M., Thone F., 1976, Histochem. J., **8**, 301.
2. Burley R., Cook M., 1961, Can. J. Biochem. Physiol., **39**, 1295.
3. Connock M. J., Sturdee A. P. 1975, Histochem. J., **7**, 103.

4. Davis J., 1964, Ann. N. Y. Acad. Sci., **121**, 494.
5. Dingle J. T., 1977, *Lysosomes*, North-Holland Pub. Comp., Amsterdam.
6. Ey P. L., Ferber E., 1977, Biochem. Biophys. Acta, **480**, 163.
7. Helenius A., Simons K., 1975, Biochim. Biophys. Acta, **415**, 29.
8. Ishiyama V., Izquierdo L., 1977, J. Embryol. exp. Morphol. **42**, 305.
9. Mellgren R. L., Slaughter G. R., Thomas J. A., 1977, J. biol. Chem., **252**, 6082.
10. Miyayama H., Solomon R., Sasaki M., Lin C. W., Fishman W. H., 1975, J. Histochem. Cytochem., **23**,, 439.
11. Sasaki M., Fishman W. H., 1973, J. Histochem. Cytochem., **21**, 653.
12. Sohn R., Marinetti G. V., 1974, Chem. Phys. Lipids, **12**, 17.
13. Wallace R., Jared D., Eisen A., 1966, Canad. J. Biochem., **44**, 1647.
14. Varo M. I., Mester R., Scripcariu D., Pecingine L., 1977, Rev. Roum. Biol.—Biol. anim. **22**, 27.

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THE ULTRASTRUCTURAL MODIFICATIONS OF ELECTRONODENSE CELLS IN THE LIVER OF *RANA ESCULENTA* L. AFTER CARBON TETRACHLORIDE ADMINISTRATION

BY

MOHAMED EL ALFY, D. MIŞCALENCU and FLORICA MAILAT

Extensive electronodense zones are present in the hepatic parenchyma in the animals treated with carbon tetrachloride and dark small spots which indicate the presence of melanocytes are disseminated in the untreated animals.

The extended electrono-opaque zones in the treated animals are constituted of groups of phagocytary elements which indicate their presence by the ingested melanocytes. They are phagocytes with ingested melanocytes and contain melanosomes in different stages of alteration.

In the cytoplasm of macrophages, in the vicinity of ingested melanocytes, we can see formations some of which look like dumbbells, with erythrocyte-like constitution.

The granule-containing cells in Amphibians' liver have been the subject of some discussions concerning their quality and functions (Allen *et al.* [3], De Robertis [5], Mişcalencu *et al.* [9]).

Andrew [2] indicated these cells as melanocytes, macrophages or epithelial cells of the hepatic parenchyma.

Roels *et al.* [11] observed that some sinusoidal cells apparently macrophages are full of melanosome- like granules.

In the case of *Ichthyophis kohtaoensis*, Welsch [14] sustains the existence of pigmentary cells which would have macrophagic functions, in Disse's space.

The present study illustrates that the treatment with carbon tetrachloride induces modifications in frogs' liver which underline the presence of both phagocytes and pigmentary cells (melanocytes) in the hepatic parenchyma.

MATERIALS AND METHODS

The animals received "per os" carbon tetrachloride in paraffine oil (10%, 0.50 cm^3). After first administration, seven from nine animals died. The two survived frogs received another dose of carbon tetrachloride at 48 hours after the first administration and were sacrificed two hours later.

The small pieces of liver were double-fixed in glutaraldehyde and osmium tetraoxide and the ultrasections were double-stained with uranyl acetate and lead citrate.

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The sections were examined by Philips electronomicroscope.

Other fragments were prepared for the light microscope (Carnoy fixation and Hemalaum Mayer stain).

RESULTS

The severe modifications which carbon tetrachloride induces in frogs' liver refer to the pigmentary cells (melanocytes). If in normal conditions the melanocytes are disseminated in the whole hepatic parenchyma, the treatment with carbon tetrachloride induced in animals a concentration of melanocytes, thus creating less but more extended pigmentary zones and with more compact pigmentary material (Fig.1).

Electron microscopy displayed these compact zones as an agglomeration of phagocytes the cytoplasm of which contains one or more cells with electronodense granules (Fig. 2).

When the ingested cells are in a less advanced stage of alteration, they contain pigmentary granules with normal features. But most of them have diminished size and electronodensity (Figs. 2, 3).

In more advanced stages of destruction the ingested cells have smaller dimensions, homogenized cytoplasm and increased electronodensity (Fig. 3). In the last phase of digestion, the ingested cells disintegrate in the cytoplasm of phagocytes but they keep some electronodense granules of the phagocyted cells. On the other hand, they can transform in a sack in which we cannot distinguish organelles, but only electronodense masses fused to each other. In this stage the phagocytes and the phagocyted cells contain electronodense granules of the phagocyted cells (Fig. 4).

All the examined sections revealed erythrocytes in the vicinity or among phagocytes (Figs. 3, 4, 5). The majority of these erythrocytes presented invaginations on their surface, which sometimes deepen so much that they can transform important zones of the cell in fringy zones with numerous cellular processes of different forms (Fig. 4). This process may advance till the transformation of erythrocytes in an amoeba-like form with numerous pseudopodia (Fig. 5). All these transformations lead to cell fragmentation till its disappearance because the processes emitted by erythrocytes detach from the cell (Fig. 5).

The phagocytes contain in their cytoplasm some formations of different aspects, but sometimes they have the typical form of dumbbells (Fig. 6). These have peripherically a double membrane and their content is homogeneously granular-like erythrocytes cytoplasm and it selectronoopacity (Fig. 7). These frequently illustrate a lysis state.

DISCUSSIONS

The toxic action of carbon tetrachloride on the frogs' hepatocytes induce well-known transformations as in mammalian hepatocytes (Scarpelli and Trump [12], Confer and Stenger [4]).

The aggregated state of melanocytes (Welsch and Storch [14]) ingested by macrophages (Andrew [1], [2], Roels, Schiller and Goldfischer

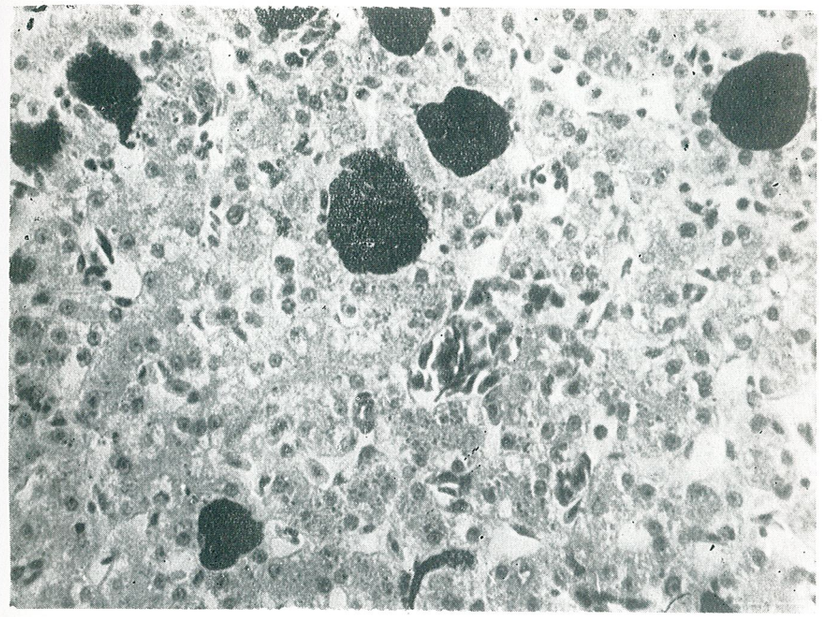

Fig. 1. — Following the treatment with carbon tetrachloride, melanocytes aggregate and constitute compact zones in the liver of *Rana esculenta*.

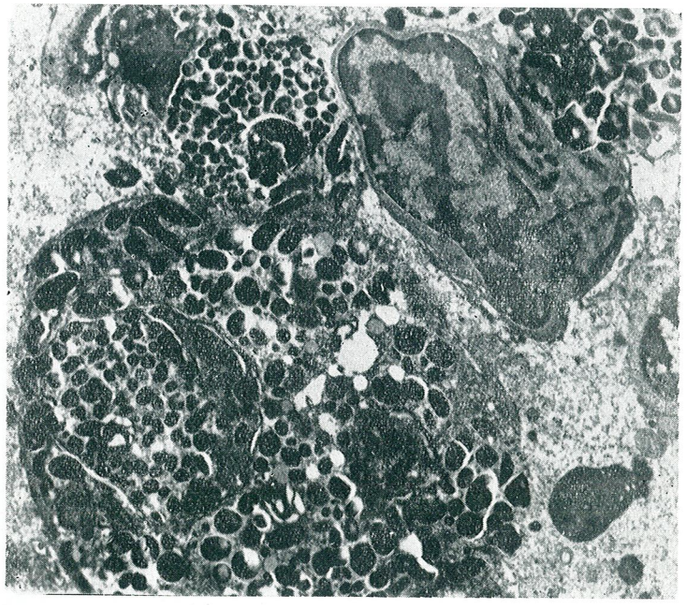

Fig. 2. — A compact zone with some macrophages which contain melanocytes in different stages of disintegration. × 5200.

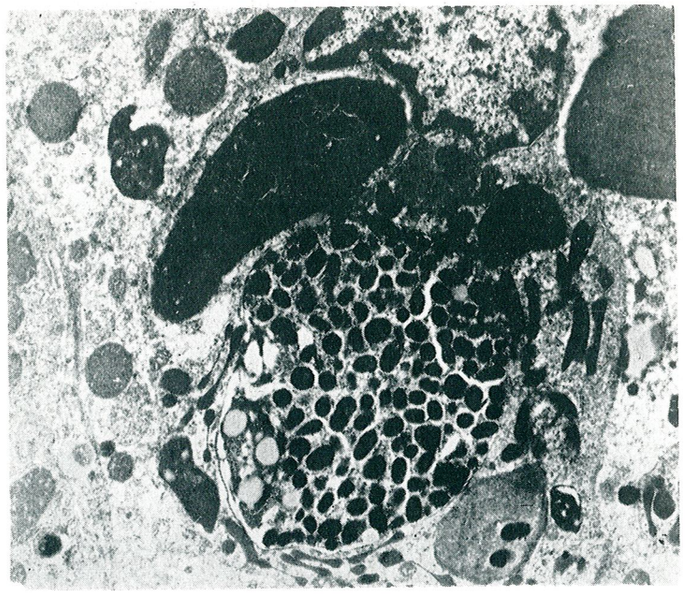

Fig. 3. — Two melanocytes in a different stage of disintegration. In the vicinity of the less advanced destroyed melanocytes there are dumbbell-like formations. On the upper right—one erythrocyte fragment. × 7 400.

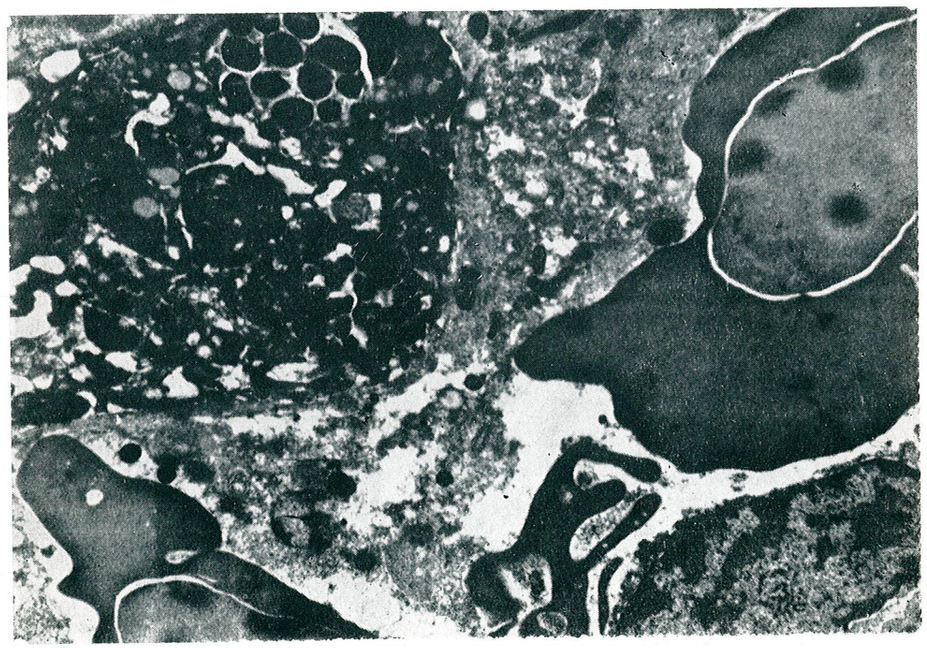

Fig. 4. — Advanced destruction of melanocytes. Between the two entire erythrocytes we can observe fringy debris of the other erythrocytes. × 8 000.

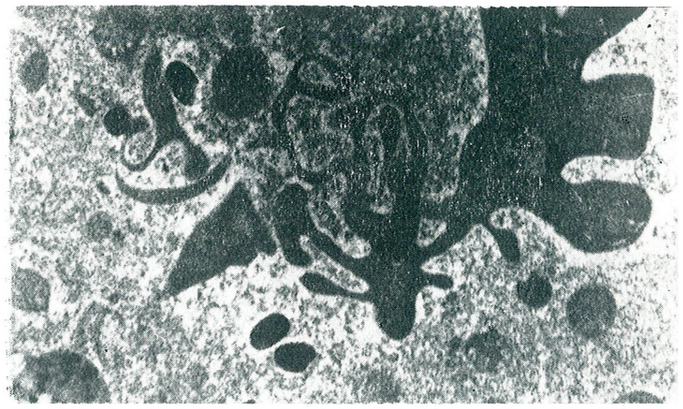

Fig. 5. — The fragmentation of one erythrocyte. × 13 000.

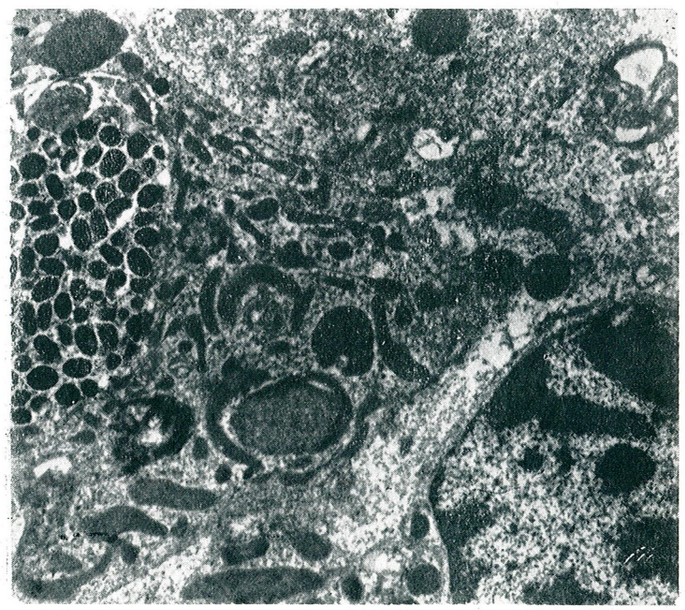

Fig. 6. — One macrophage which contains a melanocyte and some dumbbel-like formations. × 8 500.

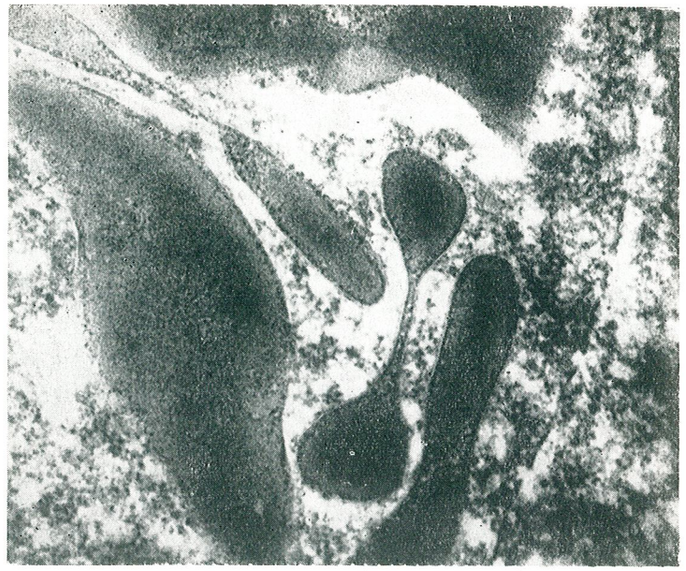

Fig. 7. — The structure of dumbbell-like formations. × 52 500.

[11]) determine those compact electronodense zones in the liver of frogs which were exposed to carbon tetrachloride treatment.

Even the hepatocytes are in an advanced stage of alteration (David [6], Mazocco [8], Nicholes, Follet and Event [10], Stenger [13]); they are not ingested by phagocytes. The examined macrophages contain only pigmentary cells (melanocytes) in their cytoplasm.

The presence of erythrocytes in this conglomerate of macropahages and melanocytes as also their fragmentation suggest that they also take part in some way to the events of this zone.

Because the dumbbells-like bodies in the cytoplasm of macrophages look like the content of erythrocytes, make us consider that these are fragments of erythrocytes ingested by macrophages. May be this ingestion take place for the digestion of erythrocytes by macrophages (Kew *et al.* [7]).

We consider that the positive reaction of macrophages for iron (Andrew [2], Kew *et al.* [7]) is due to the erythrocytes ingested.

The appearance of compact electronodense zones in the liver of *Rana esculenta* treated with carbon tetrachloride is due to the phagocytes which ingested melanocytes.

REFERENCES

1. Andrew W., 1959 *Text-book of Comparative Histology*, New York, Oxford University Press.
2. Andrew W., 1969, J. Cell. Biol., **43.**
3. Allen J. R., Carstens L. A., Norback D. H., Loh P. M., 1970, Cancer Res., **30**, 1857–1866.
4. Confer B. D. and Stenger J. R., 1966, Cancer Res., **26,** 834–843.
5. De Robertis E. D. P., 1938, C. R. Soc. Biol., Paris, **129,** 862–864.
6. David H., 1962, Z. Zellforsch., **57,** 567–571.
7. Kew M. C., Minick O. T., Bahu R. M., Stein R. J., Kent G., 1978, Am. J. Pathol., **90,** 609–618.
8. Mazocco P., 1938, C. R. Soc. Biol., Paris, **129,** 857–859.
9. Mişcalencu D., Iordăchel C. M., Mailat F., Mihăescu G., Untu C., 1978, Acta Anat., **101,** 10–18.
10. Nicholes T. J., Follet B. K., Event P. J., 1968, Z. Zellforsch, **90,** 19–27.
11. Roels F., Schiller B., Goldfischer S., 1970, Z. Zellforsch. , **108,** 135–149.
12. Scarpelli G. D., Trump F. B., 1971, *Cell Injury,* The Upjohn Company, Kalamazoo, Michigan.
13. Stenger, J. R., 1966, J. Ultrastruct. Res., **14,** 240–253.
14. Welsch U., Storch V., 1972, Zool. Jb. Anat., **89,** 621–635.
15. Widmann J. J., Cotran S. R., Fahmi H. D. 1972, J. Cell Biol., **52,** 159–170.

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EFFECTS OF THYMUS EXTRACTS ON ISOLATED FROG HEART

BY

VIRGIL TOMA and RODICA GIURGEA *

The perfusion of isolated frog heart with a homogenate of normal rat thymus shows stimulatory (6%) or inhibitory (12%) effects, depending on the concentration.
Preparations of thymus involuted by hydrocortisone, when the gland presents a hypertrophiate epithelial reticulum (hormo-secretory), have inhibitory effects on the heart frequency.

In previous papers it was shown that thymus extracts influence the activity of the cardiovascular system [2], [4], [6]. The action of these extracts depends on the functional state of the thymus. It is known in this respect that the reactivity of the gland differs function of the ontogenetic evolution as in the case of stress action factors [5].

MATERIALS AND METHODS

We used a homogenate of thymus gland taken from white Wistar female rats, weighing 100 ± 10g; then, from similar animals after 3 days of an i.m. injection of 7.5 mg hydrocortisone (CIF), i.e. when the involution effect of the hormone on the thymus is maximal [7].

The thymus gland of both groups was extracted, weighed on a torsion balance, then homogenized in a Waring-blendor, in cold Ringer solution. The obtained solutions was filtered through Watman paper no. 3, reporting the quantity of tissue on unity of serum volume. Isolated frog hearts were perfused with this extract on a frequency of 70 ± 15 drops/minute.

RESULTS AND DISCUSSION

Normal thymus extracts influence the frog heart, depending upon its concentration. So, at a concentration of 6% (60 mg fresh tissue/ml), it produces constant inotropic effects, similar to those obtained with a thymic extract CIF (1%), and partially with the extract TP/EIB (thymo proteic extract prepared within the Endocrinological Institute of Bucha-

* With the technical assistance of Dumitru Văduva.

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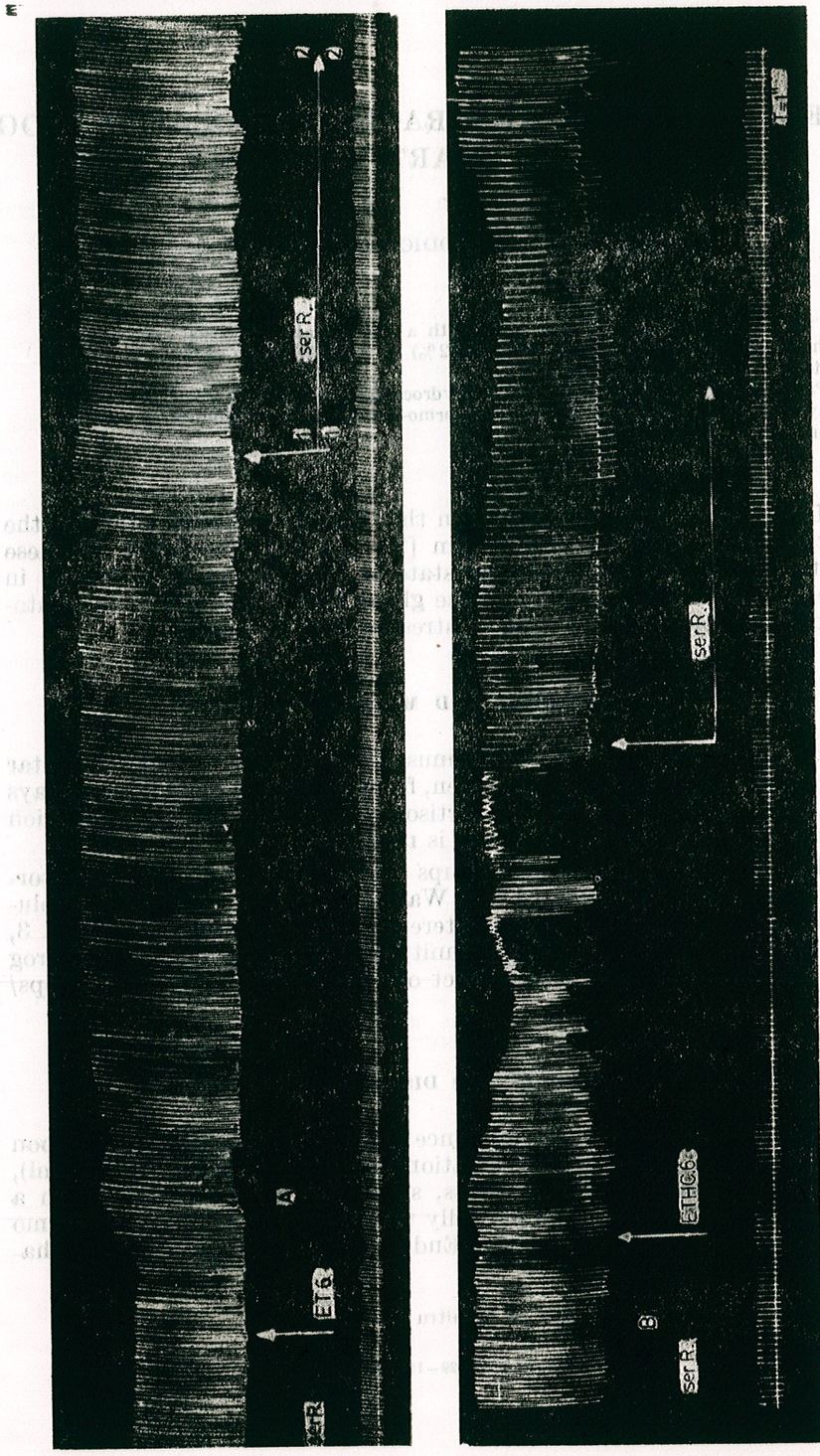

Fig. 1. — A. Cardiogram under action of normal thymus extract (6%). B. Inhibitory action of involuted thymus extract (6%), on isolated frog heart.

rest, Prof. S. Milcu and I. Potop), when perfused at a concentration of 16% [6]. Over this concentration thymic extracts produce an inhibition of the heart frequency and arhythmias which may be removed washing the heart with Ringer solution.

The extracts of thymus involuted by hydrocortisone show cardiac inhibition effects even at a concentration of 6%. The inhibition manifests itself by arhythmias associated with fibrillations and after washing with serum the activity of the heart returns to normal, but later on after a chronotropic negative phase.

These data demonstrate that the thymus contains compounds which may influence the isolated frog heart depending on their concentration. At the same time one can see that this influence is dependent upon the morpho-functional state of the gland. The effects seen on perfusion with normal thymic extract may be attributed to the lymphocytic component of thymus (T) as well as to its epithelial reticulum. In fact these cells contain active immunopeptides-lymphokines [1]. In the case of the involuted thymus, depleted of lymphocytes, the effects may be attributed to an active compound elaborated by the epithelial reticulum or the Hassal's corpuscles [3].

By using the isolated frog heart one can show the difference in activity between normal and acutely involuted thymus.

REFERENCES

1. Khan A., 1978, Ann. Allergy, **41**, 78.
2. Kocsis A., Varga E., 1942, Tisza Istv. Trs., II, oszt. munkái (Debreczen), 397.
3. Metcalf D., 1966, *The Thymus*. Springer Verlag, Berlin, Heidelberg, New York.
4. Pora A. E., Toma V. 1971, Rev. Roum. d'Endocrinol., **8**, 57.
5. Pora A. E., Toma V. 1969, Ann.Endocrin., **30**, 519.
6. Toma V., Roman H., 1969, Lucrări ştiinţifice, Inst. Ped. Oradea, 371.
7. Toma V., Giurgea R., 1974, Zbl. Vet. Med. A, **21**, 506.

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STEUERUNGSMECHANISMEN DER BLUTHOMÖORHOPIE BEI KANINCHEN

VON

HORST KOLASSOVITS

Mechanisms of regulation of blood homeorhopy were studied in rabbits. Immediate and highly efficient local regulation is then supported by slower hypothalamic processes. These were put into evidence by sham-disturbance of ionic equilibrium of the blood (ionic solutions microinjected in the anterior hypothalamic area). Excess of K^+ is more rapidly eliminated from the plasma than excess of Ca^{2+}. The latter may also lead to a disturbance of the homeorhopic capacity of the organism.

Ausgehend von dem Prinzip der „Pseudoinformation" durch Ioneneinwirkung auf den vorderen Hypothalamus (AHA), wo Ionenrezeptoren bekannt sind [4], [5], führt die vorliegende Arbeit einige Aspekte der rhopischen Regelung der Blutzusammensetzung an [7].

Als Versuchstiere dienten Chinchilla-Kaninchen beiderlei Geschlechts, mit einem durchschnittlichen Gewicht von 3,2 kg. Unter Nembutalanästhesie (i.p. 45 mg/kg-Körpergewicht) wurden zweierlei Versuchsreihen durchgeführt:

1) um festzustellen wie der Gesamtorganismus normalerweise die Rhopie des Blutes regelt, wurden i.v. isotonische Lösungen (338 mOsM von KCl 12,2‰ und $CaCl_2$ 12,9‰) nach halbstündiger Anästhesie in solchen Mengen verabreicht, daß der Kaliumgehalt des Blutplasmas verdoppelt (2 × K) oder der Kalziumgehalt verdreifacht (3 × Ca) wurde. Sofort wurden anschließend Blutproben (je 4 cm^3) aus der Ohrrandvene entnommen, heparinisiert und folgende Blutkonstanten bestimmt: Wassergehalt (gravimetrisch); Proteine (refraktometrisch); Hematokrit; Natrium-, Kalium-, Kalziumkonzentrationen im Blut und Plasma (flammenfotometrisch). Die Vergleichsproben wurden vor dem Anästhesieren, bzw. vor der Einspritzung entnommen, die restlichen Blutproben während 90 Minuten in immer größeren Abständen.

2) um festzustellen wie der Hypothalamus in die Regelung der Blutrhopie eingreift, wurden 2 mm^3 isosmotisches, polysalines Serum (NaCl, KCl, $CaCl_2$) in die AHA (Stereotaxis-Atlas [11], Stereotaxisgerät MB-4001, Hohlnadel ∅360 μ) eingespritzt. Für die Kontrollgruppe waren die Ionenverhältnisse des Serums identisch mit denen des Normalblutes (Vergleichsproben), für andere Tiergruppen war des K/Ca-Verhältnis vergrößert bzw. verkleinert. Postexperimentell wurden durch die unbeweget gebliebene Nadel 2 mm^3 Chinatusche injiziert, um durch histologische Kontrolle die genaue Einspritzstelle leichter zu ermitteln.

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Die Ionenkonzentration wurde in mg/100 cm³ Plasma, die Plasmaionenmenge in mg Plasmaionen in 100 cm³ Blut ausgedrückt. Statistisch signifikant sind die Ergebnisse bei $P \leq 0{,}05$.

VERSUCHSERGEBNISSE

Die Nembutalanästhesie vermindert statistisch signifikant bloß den Protein-und Kaliumgehalt des Blutes (Abb. 1) [1]. Es sei auch erwähnt, daß durch wiederholten Blutentzug ungefähr nach 10 Minuten eine Verdünnung des Blutes auftritt [3], die hier nicht näher besprochen wird. Die Auswirkungen dieser beiden Tatsachen wurden jedoch in allen Berechnungen der Ionenzusammensetzung des Blutes berücksichtigt.

Gleich nachdem die Salzlösung (2 × K; 3 × Ca) ins Blut gelangt, steigt die jeweilige Kationenkonzentration sehr rasch an und fällt für K in ungefähr 5 Minuten und für Ca in ungefähr einer Stunde auf die Ausgangswerte zurück. Die prozentuellen Veränderungen der Plasma-Ionenmenge im Blut nach i.v. Einspritzen isotonischer KCl-Lösung (2 × × K) sind aus Abb.2 ersichtlich, während Abb. 3 den Tatbestand beim

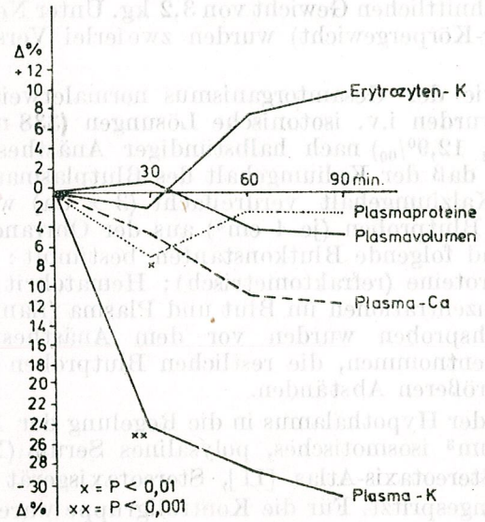
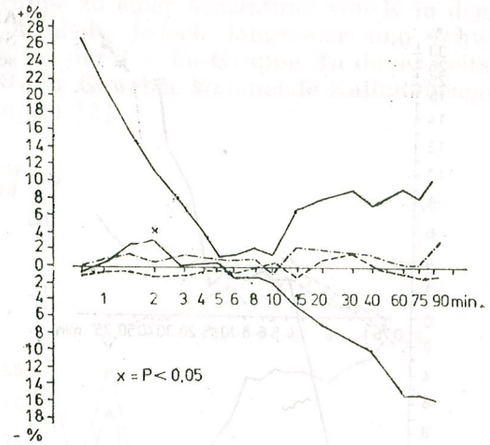


Abb. 1 — Veränderungen einiger Blutkomponenten durch Nembutalanästhesie. Abszisse: Zeitangabe seit Anästhesieeintritt. Ordinate: Veränderungen in Prozenten der Anfangswerte (bei nicht anästhesierten Tieren). Kalium-und Kalziummengen in 100 cm³ Blut.

i.v. Einspritzen von isotonischer $CaCl_2$-Lösung (3 × Ca) angibt. Die prozentuellen Veränderungen des Plasma-Ionengehaltes im Blut nach dem Einspritzen eines isosmotischen Serums mit erhöhtem Kaliumgehalt in die AHA ist in Abb. 4 dargestellt. Abb. 5 zeigt die Ergebnisse nach dem Verabreichen in die AHA von Serum mit erhöhtem Kalziumgehalt.

Abb. 2. — Änderung des Gehaltes von K, Ca und Na im Blutplasma und an K in den roten Blutkörperchen in 100 cm³ Blut, in Prozenten der Anfangswerte. Intravenöse Injektion von isotonischer KCl-Lösung (2 × K-Gruppe). —·—·— = Plasma—Na, die übrigen Zeichenerklärungen siehe Abb. 1.



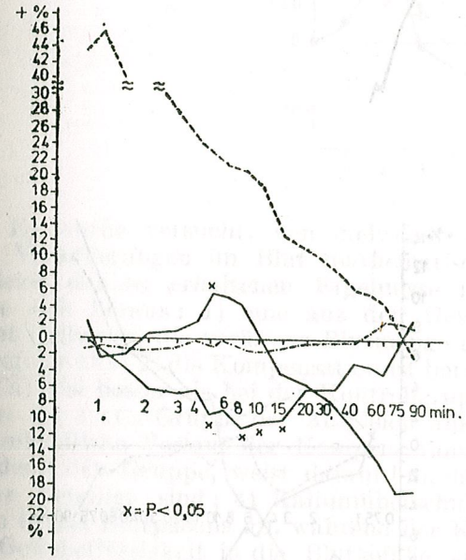


Abb.3. — Intravenöse Injektion von isotonischer $CaCl_2$-Lösung (3 × Ca-Gruppe). Andere Erklärungen siehe Abb 2.

DISKUSSION DER VERSUCHSERGEBNISSE

Der größte Teil (80%) des i.v. eingespritzten Ionenüberschusses verschwindet in der ersten Minute (erste Phase), wahrscheinlich durch Ablagerung in den Gefäßwänden. Derart rascher Konzentrationsabfall ist bekannt für Na [9], [10] für K [8], jedoch nicht für Ca. Im Verlauf der nächstfolgenden zwei Minuten, also zu Beginn der zweiten Phase,

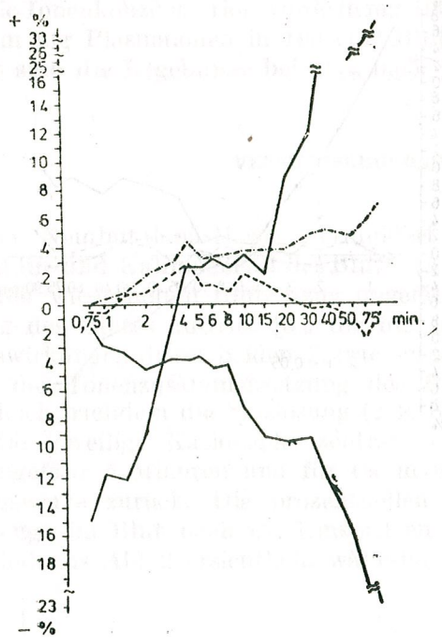


Abb. 4. — Injektion von Serum mit Kaliumüberschuß in die AHA. Andere Erklärungen siehe Abb.2.

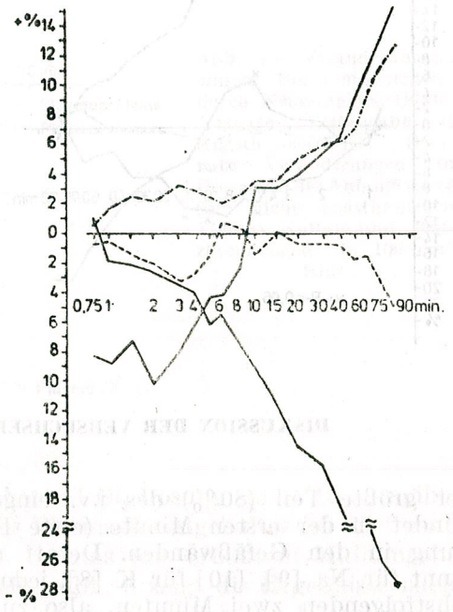


Abb.5. — Injektion von Serum mit Kalziumüberschuß in die AHA. Andere Erklärungen siehe Abb. 2.

kommt es bei der 2 × K-Gruppe zu einer Anhäufung von K in den roten Blutkörperchen (Abb. 6). Ähnlich, jedoch langsamer und schwächer, verlaufen die Vorgänge auch bei der 3 × Ca-Gruppe. In dieser Zeitspanne dringt auch eine neue, aus den Geweben kommende Kaliummenge kurzfristig in die Blutkörperchen ein [2].

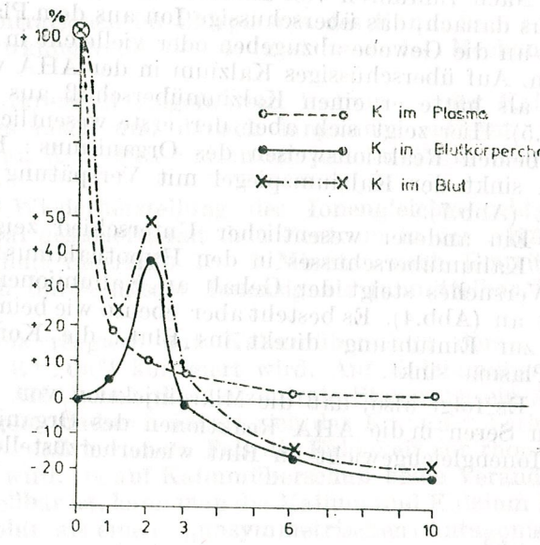


Abb.6. — Prozentuelle Änderung der Kaliummenge in einem bestimmten Blutvolumen innerhalb 10 Minuten nach der Injektion, bezogen auf die ursprünglich vorhandene Menge. Versuchsgruppe 2 × K.

Es wurde versucht, von mehreren Arbeitshypothesen ausgehend diese Veränderungen im Blut mathematisch zu erfassen [2] [3]. Beim Vergleich der so erhaltenen Ergebnisse mit den Versuchsergebnissen, stellte sich heraus: 1) eine aus den Geweben stammende Flüssigkeit gleicht (teilweise) die verlorene Blutmenge und auch die verlorenen Plasmaproteine aus; 2) die Kompensation ist bei den Versuchsgruppen (2 × K; 3 × Ca) viel besser als bei der Kontrollgruppe (in den ersten 10 Minuten ist bei der 3×Ca-Gruppe der Ausgleich anscheinend vollständig); 3) der unterschiedliche Verlauf der Kompensationskurven bei der 2xK-Gruppe und der 3×Ca-Gruppe, weist darauf hin, daß dabei verschiedene Mechanismen beteiligt sind; 4) Kaliumüberschuß regt den Eintritt von Proteinen in das Blutplasma an, während der Kalziumüberschuß den Eintritt von Gewebeflüssigkeit in die Blutgefäße verzögert; somit führen beide Störungsarten des K/Ca-Verhältnisses zu einer Verminderung der Blutverdünnung als Folge der Blutung; 5) durch Hämorrhagie bewirkter Ionenverlust, besonders von K wird auch teilweise ausgeglichen; bei der 2×K-Gruppe stammt das Kalium hauptsächlich aus den Geweben und bei der 3×Ca-Gruppe aus den roten Blutkörperchen.

Auf die Rolle des Hypothalamus bei der Aufrechterhaltung des Ionengleichgewichtes im Blut machte Popa aufmerksam [6]. Die ersten uns bekannten systematischen Untersuchungen dazu sind die Arbeiten von Pop [4], [5].

Unsere Ergebnisse beweisen, daß die AHA das Ionengleichgewicht im Plasma beeinflußt. Mikroinjektionen von isotonischem Serum mit Kalium-oder Kalziumüberschuß in die AHA wirkten auf den Kalium- bzw. Kalziumspiegel ähnlich (doch weder quantitativ noch qualitativ gleich) wie die Einführung der Ionen direkt in den Blutkreislauf.

Nach Einführen von Kalzium in den Blutkreislauf strebt der Organismus danach, das überschüssige Ion aus dem Plasma zu entfernen (Abb. 3), es an die Gewebe abzugeben oder vielleicht in den Gefäßwänden abzulagern. Auf überschüssiges Kalzium in der AHA verhält sich der Organismus, als hätte er einen Kalziumüberschuß aus dem Blut zu entfernen (Abb.5). Hier zeigt sich aber der erste wesentliche Unterschied zwischen den beiden Reaktionsweisen des Organismus: bei Verabreichen in die AHA sinkt der Kalziumspiegel mit Verspätung und ist weniger ausgeprägt (Abb.7).

Ein anderer wesentlicher Unterschied zeigt sich beim Einführen eines Kaliumüberschusses in den Hypothalamus: in den ersten Minuten des Versuches steigt der Gehalt an Kaliumionen in den Blutkörperchen nicht an (Abb.4). Es besteht aber ebenso wie beim Kalzium eine Ähnlichkeit zur Einführung direkt ins Blut: die Konzentration des Kaliums im Plasma sinkt.

Es folgt also, daß die Mikroinjektion von unausgeglichenen rhopischen Seren in die AHA Reaktionen des Organismus bewirkte, als wäre das Ionengleichgewicht im Blut wiederherzustellen. Also ist anzunehmen,

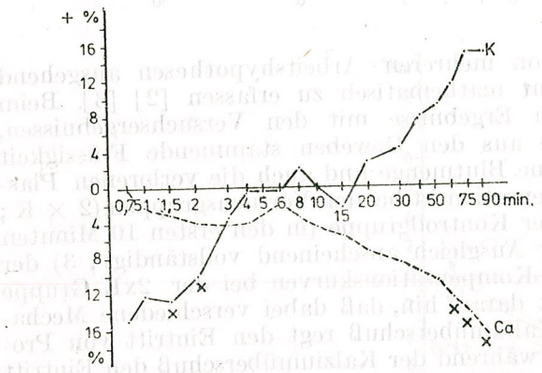


Abb.7. — Änderung der Konzentrationen von K und Ca im Plasma nach Injektion von Serum mit K-bzw. Ca-Überschuß in die AHA. Prozente der ursprünglichen Werte.

daß die AHA die Ionenkonzentration im Blut steuert, daß es hier ein Zen trum gibt, für das die Ionenkonzentrationen im durchströmenden Blut plasma Informationen darstellen. Änderungen dieser ununterbrochenen Reize, die tatsächlichen Veränderungen in der Zusammensetzung de Blutes entsprechen oder (bei den Injektionen in die AHA) solche vortäu schen, sind „Ionensignale" für diesbezüglich spezialisierte AHA-Rezep torzellen. Die AHA steuert diese Vorgänge wahrscheinlich durch eine neurosekretorischen Mechanismus, der spät einsetzt, aber andauert, sowi durch einen rasch einsetzenden parasympathischen Mechanismus. Letz

terer bewirkt — unter anderem — durch die Absonderung von Acethylcholin in den gesamten Kreislauf einen plötzlichen und vorübergehenden Anstieg der Membrandurchlässigkeit der roten Blutkörperchen.

SCHLUSSFOLGERUNGEN

1. Die Ionenkonzentrationen im Blutplasma des Kaninchens werden durch prompte und wirkungsvolle (lokale und zentrale) Mechanismen gesteuert.

2. Die in den Blutkreislauf eingeführten Kalium — oder Kalziummengen werden anfangs rasch und danach langsamer entfernt, wobei der restliche Überschuß an K in etwa 5 Minuten, jener an Ca in etwa einer Stunde verschwindet.

3. Die langsame Wiederherstellung des Ionengleichgewichtes im Plasma ist neuro-humoral gesteuert, mit dem Zentrum in der AHA. Die zentrale Steuerung beginnt innerhalb 1 — 2 Minuten nach Eintritt der Störung. Sie kann auch durch falsche Ionensignale (unmittelbar in die AHA) ausgelöst werden.

4. Der Organismus reagiert auf Kaliumüberschuß derart, daß das gestörte Verhältnis $K^+ : Ca^{2+}$ korrigiert wird. Auf Kalziumüberschuß hingegen (im Blut oder AHA), bewirkt die zentrale Steuerung ein Absinken des Plasma-K-Spiegels; dadurch ändert sich das $K^+ : Ca^{2+}$ Verhältnis noch mehr. Daraus folgt, daß in diesem Fall die Fähigkeit zur rhopischen Steuerung selbst gestört wird. Da auf Kaliumüberschuß keine Veränderung im Ca-Blutspiegel feststellbar ist, kann man die Kalium und Kalzium Beziehungen im Kaninchenblut als einen „unsymmetrischen Antagonismus" betrachten.

BIBLIOGRAPHIE

1. Kolassovits H., 1973, St. și cerc. biol., Seria zool., **25**, *3*, 253—256.
2. Kolassovits H., 1974, St. și cerc. biol., Seria zool., **26**, *2*, 121—132.
3. Kolassovits H., Pora E. A., Wittenberger C., 1973, St. și cerc. biol., Seria zool., **25**, *2* 155—162.
4. Pop M., 1968, „Dissertation", Cluj.
5. Pop M., Pora E. A., Filipaș I., 1966, Studia Univ. Babeș-Bolyai, Ser. biol., *2*, 137—143.
6. Popa G. T., 1939, Acad. Rom., Mem. secț. științ., Ser. III, tom XIV, Mem. *11*, 221—252.
7. Pora E. A., 1958, Proceed. XV. Intern. Congr. Zool., Lond. 1061—1063.
8. Pora E. A., Stoicovici F., 1964, Rev. Roum. Biol., Series de zool., **9**, *4*, 257—269.
9. Pora E. A., Roșca D. I., Wittenberger C., Stoicovici F., Rușdea D., 1960, Comun. Acad. RPR, **10** 839—846.
10. Ripplinger J., Mallie J. P., 1961, C. R. Soc. Biol., **155**, 2371—2374.
11. Sawyer Ch., Everett J., Green J., 1954, J. Compar. Neur. **101**, *3*.

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EFFECT OF ACUTE STRESS-STIMULUS UPON THE INSULIN SENSITIVITY OF WHITE RATS DURING EARLY POSTNATAL ONTOGENY

BY

I. MADAR, NINA ŞILDAN, ANA ILONCA and E. A. PORA

The insulin sensitivity of control and stressed suckling (15-day-old) and weanling (20-25-day-old) rats on the basis of intravenous insulin tolerance test was estimated. It was established that in the control animals the sensitivity to insulin enhances with advancing in age, and that in weanling rats (but not in sucklings) 14 hours after stress-induction (produced by a subcutaneous injection of a single dose of 0.5 ml 2% formaldehyde/100 g.b.w.) there appear a significant decrease in insulin responsiveness.

It is well established that in white rats there is a causal relationship between the degree of stress-induced glucocorticoid excess and insulin resistance [13]. On the other hand, it has been demonstrated that formaldehyde intoxication, by activation of the pituitary-adrenocortical axis, is a potent stressor in inducing a relatively persistent glucocorticoid excess [5], [11], [13] and insulin resistance in rats [13]. Furthermore, the behavioral literature indicates that there are age-related differences in mature and infant metabolic and endocrine responses to the same stressor [12].

Taking into consideration the above mentioned facts as well as that in the functional maturation of pituitary-adrenocortical system of white rats there are critical stages during early postnatal development [4], [9], the aim of the present work was to compare the age-related dynamics of insulin sensitivity of control and stressed young rats, using the intravenous insulin tolerance test as a sensitive index of the antiinsulinic effect of glucocorticoid excess [7].

MATERIALS AND METHODS

The experiments were carried out on suckling (15-day-old) and weanling (20-and 25-day-old) Wistar rats, from the stockfarm of our laboratory. The animals were kept at a constant room temperature (24°C). Excepting the suckling rats, the others were fed additionally with a dry mixed laboratory diet ("RATIFORT") in which 60% of calories were supplied by carbohydrates, 25% by proteins and 15% by fats, drinking water being provided *ad libitum*.

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Prior to the experiments, the animals were starved 14 hours. Acute stress-stimulus was applied 14 hours before experiments by injecting a single dose of 0.5 ml diluted formaldehyde (2%) per 100 g b.w. into the subscapular skin fold, while controls received an equal volume of saline.

In both controls and stressed rats intravenous insulin tolerance test was performed under Nembutal anaesthesia, by injecting a dose of 2×10^{-2} units recrystallized ox-insulin ("Calbiochem", San Diego, Calif., Lot 201248, Gerade B, 26.1 I.U./mg) per 100 g b.w., through one of the tail veins. The insulin was diluted in Krebs-Henseleit buffer, containing 1% bovine serum albumine ("Serva").

Blood samples for glycemia were taken from the tail vessels both initially (10 minutes after anaesthesia) and 20-minute intervals during 60 minutes after insulin administration. Glycemia was determined from 50 microliters blood using the glucoseoxidase-peroxidase method of Krebs *et al.* [6], and expressed as mg glucose/100 ml blood. The optical density of the samples was read at 545 nm, using a spectrophotometer ("Spekol", Carl Zeiss, Jena).

On the basis of semilogarithmic representation of the hypoglycemia curves as well as of the insulin activity curves, the mathematical evaluation of the insulin sensitivity was made by calculating the insulin activity coefficients (i), using the formula of Franckson [3] and the procedure of Bellens [1].

The statistical treatment of the data was performed according to the Student t test, P values of 0.05, or less, being considered significant.

RESULTS AND DISCUSSIONS

As one can see from the data summarized in table 1 and figure 1, the sensitivity of control rats to exogenous insulin is strongly age-related. Thus, in the suckling animals (15-day-old- ones) the rate of insulin-promoted glucose penetration from the blood into the tissues is relatively low, the mean value of insulin activity coefficients (i) being equal to 2.347. In comparison with this, the insulin sensitivity of weanling rats is significantly enhanced, but the rate of blood glucose decrease under the effect of hormone in 25-day-old animals is more accentuated than in 20-day-old ones ($i = 4.702$ *vs* 3.505, $P < 0.001$). These data suggest that during the studied ontogenetical period of rats the maturation of insulin-sensitive glucose transport system advances with age. At the same time, the above results give support to the evidences [2] that transition of rats at weaning from maternal milk to a rich carbohydrate containing mixed laboratory diet induces an abrupt increase in both insulin responsiveness of the organism and insulin secreting capacity of the endocrine pancreas.

When intravenous insulin tolerance test is performed 14 hours after sress-induction by formaldehyde, in the suckling rats the sensitivity to insulin remains unchanged as compared to the corresponding control values. On the contrary, in the weanling rats (20-and 25-day-old ones) 14 hours following stress stimulus the insulin sensitivity is markedly decreased ($i = 2.355$ *vs.* 3.505, and $i = 3.117$ *vs.* 4.702, respectively,

Table 1

The parameters of the intravenous insulin tolerance in various age-groups of control and stressed rats. C_0=initialglycemia (mg/glucose/100 ml blood); C_{20}, C_{40} and C_{60}= glycemia levels at 20,40 and 60 minutes, respectively, after intravenous injection of 2×10^{-2} units of insulin/100 g b.w.; i = insulin activity coefficient

Control rats			
Age:	15-day-old ($n = 8$)	20-day-old ($n = 8$)	25-day-old ($n = 9$)
C_0	98±3.42	92±3.10	96±2.56
C_{20}	74±2.97	65±2.46	61±1.92
C_{40}	60±2.48	50±1.89	47±0.79
C_{60}	80±1.83	74±3.10	69±1.98
i	2.347±0.175	3.503±0.156[a]	4.702±0.224[a]
Stressed rats			
Age:	15-day-old ($n = 8$)	20-day-old ($n = 8$)	25-day-old ($n = 9$)
C_0	100±3.53	103±2.53[b]	107±2.63[b]
C_{20}	75±3.52	76±2.05	74±2.33
C_{40}	63±3.56	62±1.60	58±1.93
C_{60}	85±3.80	86±2.71	95±2.68
i	2.207±0.151	2.355±0,120[c]	3.117±0.136[c]

Results are expressed as means ± S.E. Number of experiments (n) is given in parentheses. [a]) $P < 0.001$ *vs.* 15-day-old controls; [b]) $P < 0.05$ and [c]) $P < 0.001$ *vs.* corresponding controls, respectively.

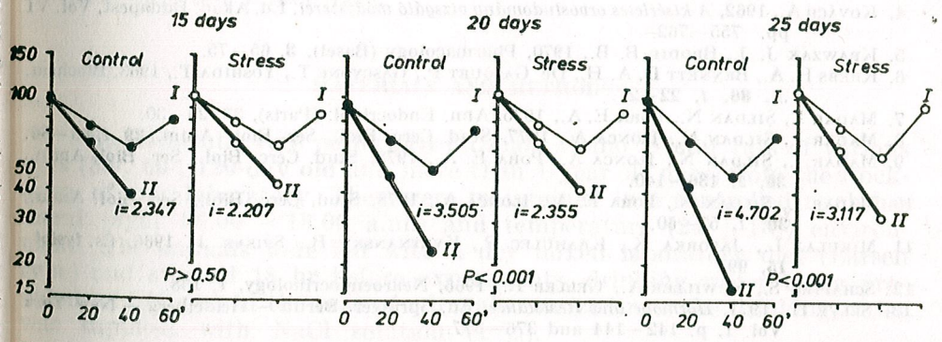

Fig. 1. — Semilogarithmic representation of intravenous insulin-hypoglycemia curves (I) and of insulin activity curves (II) in various age-groups of control and stressed rats. The curves are plotted on the basis of mean glycemia values (summarized in table 1). Blood glucose levels are given in mg/100 ml on the ordinate while blood sampling intervals in minutes are given on abscissa. i represents mean values of insulin activity coefficients.

$P < 0.001$), whereas the levels of initial glycemia are moderately, but statistically significantly enhanced ($P < 0.05$).

It should be underlined that both moderate fasting hyperglycemia and reduced insulin sensitivity of white rats after glucocorticoid administration [7] or after formaldehyde-induced stress event [13] are in close relation to the antiinsulinic effect of a relative persistent glucocorticoid excess. On this basis, as well as on the basis of our present data it is pertinent to assume that in fasting hyperglycemia and in insulin resistance of weanling rats the stress-induced glucocorticoid excess is mainly involved, and that weaning period of rats represents a critical stage in the functional maturation of pituitary-adrenocortical axis. It seems very likely that at this ontogenetical stage the antiinsulinic effect of released glucocorticoid [8], [10] and other hyperglycemic factors in response to insulin-hypoglycemia [7], may be substantially potentiated on the background of stress-induced glucocorticoid excess. In this context, the present data not only extend the knowledge about the ontogenetical aspects of the stress mechanism, but the experimental models used here appear to provide a useful tool for testing age-related metabolic and hormonal stress responses during early postnatal development of rats.

CONCLUSIONS

1. Insulin sensitivity of white rats during early postnatal ontogeny is strongly age-related.

2. Weaning period of white rats represents a critical stage in the appearance of stress-induced insulin resistance and fasting hyperglycemia.

REFERENCES

1. Bellens R., 1961, Acta Endocrinol. (Kbh.), Suppl. 61, p. 21.
2. Foà P., P., Blazquez E., Sodoyez J. C., Sodoyez-Goffaux F., 1976, In: *The Evolution of Pancreatic Islets*. (Eds. T. Adesanya, I. Grillo, L. Leibson, A. Apple) Pergamon Press, Oxford — New York — Toronto — Sydney — Paris — Frankfurt, pp. 7—18.
3. Franckson J. R. M. 1958, *Mesure de l'activité de l'insuline chez l'homme. Analyse de l'épreuve d'hypoglycémie*. I-re éd., Bruxelles.
4. Kovách A., 1962, *A kisérletes orvostudomány vizsgáló módszerei*. Ed. Akad. Budapest, Vol. VI pp. 755—762.
5. Krawzak J. J., Brodie B. B., 1970, Pharmacology (Basel), **3**, 65—75.
6. Krebs H. A., Bennett D. A. H., De Gasquet P., Gascyone T., Yoshida T., 1963, Biochem. J., **86**, *1*, 22—27.
7. Madar I., Şildan N., Pora E, A., 1975, Ann. Endocrinol. (Paris), **35**, 25—30.
8. Madar I., Şildan N., Ilonca A., 1977, Stud. Cerc. Biol., Ser. Biol. Anim., **29**, *1*, 63—66.
9. Madar I., Şildan N., Ilonca A., Pora E. A., 1978, Stud. Cerc. Biol., Ser. Biol. Anim. **30**, *2*, 136—140.
10. Madar I., Şildan N., Pora E. A., Ilonca A., 1978, Stud. Cerc. Biol., Ser. Biol. Anim. **30**, *1*, 57—60.
11. Mikulaj L., Javorka K., Krahulec P., Kvetnansky R., Spisak J., 1966, Čs. fysiol. **15**, 99.
12. Schapiro S., Yuwiller A., Geller E., 1966, Neuroendocrinology, **1**, 138.
13. Selye H., 1971, *Hormone and Resistance*, Ed. Springer, Berlin — Heidelberg — New York Vol. 1, p. 142—144 and 376—377.

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THE EFFECT OF ADRENALECTOMY AND HYDROCORTISONE UPON PENTOBARBITAL-INDUCED HYPERGLYCEMIA IN WHITE RATS OF VARIOUS AGES

BY

I. MADAR, NINA ŞILDAN, ANA ILONCA and E. A. PORA

The effect of sodium pentobarbital anaesthesia upon the dynamics of glycemia in intact, adrenalectomized and hydrocortisone-injected rats of various ages was studied. It has been established that in intact animals during short-term pentobarbital anaesthesia the fasting blood glucose level was significantly raised. Hydrocortisone hemisuccinate, depending on the age of animals, was found to potentiate the hyperglycemic effect of the anaesthetic. When pentobarbital was administered on the background of adrenalectomy, the anaesthetic failed to affect glycemia.

Sodium pentobarbital as anaesthetic drug is widely used in the exploration of carbohydrate metabolism in rats. There are numerous data indicating that this anaesthetic affects the regulatory systems involved in carbohydrate metabolism and blood glucose homeostasis in white rats [1], [4], [6] and that its effects largely depend on the duration of anaesthesia [1], [5] and on the hormonal and nutritional state of the organism [1] — [2], [5].

At present it is not clarified whether the age of intact, adrenalectomized and glucocorticoid treated rats may influence or not the glycemic response to sodium pentobarbital. Therefore, the present work was designated to investigate the age-related dynamics of glycemia during short-term pentobarbital anaesthesia in intact, adrenalectomized, and hydrocortisone-injected intact rats.

MATERIALS AND METHODS

Experiments were performed on male albino Wistar rats of various ages (30-, 60-, 120-day old and more than 1-year old ones) from the stock-farm of our laboratory. They were maintained in a controlled nychthemeral (light 06.00 — 18.00 a.m.) and temperature (24 ± 1°C) environment. The animals were fed with a dry mixed laboratory diet (Larsen type) and starved 18 hr before experiments, drinking water being provided *ad libitum*. In the case of adrenalectomized rats the drinking water was supplied with NaCl solution (1 %).

Bilateral adrenalectomy was performed by the dorsal approach, 7 days prior to experiments.

In both intact and adrenalectomized animals blood glucose was assayed in basal state, as well as 10 minutes after intraperitoneal administration of sodium pentobarbital ("Serva," 5 mg/100 g b.w.). Additionally, in intact animals glycemia was followed also 60 minutes after injection of the anaesthetic, i.e. 15 minutes after administration of a single dose of hydrocortisone hemisuccinate ("Biofarm", 0.5 mg/100 g b.w., intraperitoneally).

In order to overcome the daily rhythm of glycemia variations [7] and the diurnal variations in sensitivity to pentobarbital of rats [16], all experiments were always carried out at 08.00 — 10.00 a.m.

For testing glycemia, 50 microliters of blood samples were taken from the tail vessels, using our sampling procedure [10]. Blood glucose was assayed according to the glucoseoxidase method of Krebs *et al.* [8]. The optical density of samples was read at 545 nm, using a "Spekol" spectrophotometer (Carl Zeiss, Jena).

Glycemia values were expressed as mg glucose/100 ml blood. Mean glycemia levels were calculated in each age-group and tested for statistical homogeneity according to the criterium of Chauvenet. Differences between mean values at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

The data summarized in table 1 indicate that under basal conditions the fasting glycemia in various age-groups of intact rats are nearly the same. Ten minutes following pentobarbital administration in these animals the glycemia markedly increases as compared to the corresponding basal values. Thus, in 30-day old animals the anaesthetic induces a strong increase of the blood glucose level (63.9%, $P < 0.001$), whereas in other age-groups its hyperglycemic effect varies between 18.2 and 21.4 % ($P < 0.05$ and $P < 0.01$, respectively).

The above results are in good agreement with the observations which indicate that during pentobarbital anaesthesia in intact rats the hepatic glycogenolysis is stimulated [3], the glucose uptake by the striated muscles is diminished [2], [4] and the hypoglycemic effect of insulin is attenuated [1]. On the contrary, other experimental data indicate that in fasting rats short-term pentobarbital anaesthesia does not affect either glycemia or insulinogenic response of endocrine pancreas, while in non fasted animals this anaesthetic agent induces a hyperglycemia and a substantial fall in blood insulin level [1].

As can be seen from table 2, when sodium pentobarbital anaesthesia is induced on the background of bilateral adrenalectomy, the glycemic levels remain unchanged as compared to the basal values. This fact suggests that in intact animals the activation of the adrenals plays a major role in pentobarbital-induced elevation of glycemia. Since there are considerable data that during pentobarbital anaesthesia the basal secretion of ACTH and corticosterone in intact rats is not affected [5], we assume that in the hyperglycemic effect of sodium pentobarbital in normal animals the activation of the adrenal medulla, i.e. an enhanced adrena-

catecholamine secretion is mainly involved. It is well established that under such hormonal state, in white rats, hyperglycemia is in a close relationship to the diminution of insulin secretion [9], elevation of hepatic glycogenolysis [17] and glucose production [9], as well as to an enhanced free fatty acid release [19], [20] which, in turn, attenuates the utilisation of blood glucose by muscles [15] and other peripheral tissues.

Table 1

The mean values ± S.E. of fasting blood glucose levels in various age-groups of male albino Wistar rats under different experimental conditions. B = in basal state; SP = 10 minutes after sodium pentobarbital administration; SP + HC = 60 minutes after administration of sodium pentobarbital and 60 minutes after injection of hydrocortisone hemisuccinate, respectively

Age (days)	mg glucose /100 ml blood		
	B	SP	SP + HC
30	72 ± 3.6 (12)	118 ± 3.9 (12)	115 ± 8.0 (12)
		+63.9 % $P < 0.001$*	−2.5 % $P > 0.50$**
60	70 ± 5.1 (13)	89 ± 2.4 (13)	104 ± 1.9 (13)
		+21.4 % $P < 0.01$*	+17.1 % $P < 0.05$ **
120	75 ± 2.9 (10)	91 ± 5.5 (10)	119 ± 4.3 (10)
		+21.3 % $P < 0.01$*	+30.8 % $P > 0.01$**
365	77 ± 1.9 (9)	91 ± 5.8 (9)	93 ± 3.5 (9)
		+18.2 % $P < 0.05$*	+2.2 % $P > 0.50$ **

*) SP compared with B; ** SP+HC compared with SP. Number of experiments is given in parentheses.

Figure 1 indicates that sodium pentobarbital in 120-day old intact animals induces a relatively constant hyperglycemia during 60 minutes. This observation as well as our previous finding that hydrocortisone hemisuccinate may induce rapid modifications both in glycemia and glucose utilisation depending on the age of rats [11] — [12] by modifying the hyperglycemic effect of adrenal catecholamines [10], lead us to examine the action of this glucocorticoid upon pentobarbital-induced hyperglycemia.

Table 2

The mean values ± S.E. of fasting glycemia levels in bilaterally adrenalectomized various age-groups of albino Wistar rats under basal condition (B) and at 10 minutes after sodium pentobarbital administration (SP)

Age (days)	mg glucose/100 ml blood	
	B	SP
30	58 ± 5.6 (11)	57 ± 6.5 (11)
	−1.8 % $P > 0.50$*	
60	62 ± 4.0 (11)	65. ± 5.6 (11)
	+ 4.8 % $P > 0.50$*	
120	67 ± 2.7 (10)	71 ± 3.7 (10)
	+5.9 % $P > 0.50$	
365	69 ± 3.8 (8)	75 ± 5.4 (8)
	+8.7 % $P > 0.05$	

* SP compared with B. Number of experiments is given in parentheses.

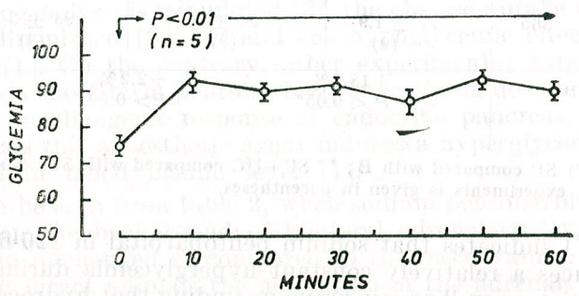

Fig. 1. — Glycemia levels (mg glucose/100 ml blood) in 120-day old Wistar rats in basal state (0 min) and at different intervals of time (10 — 50 min) during pentobarbital-induced anaesthesia. Each point and vertical line represents the mean ± S.E. obtained in 5 rats ($n = 5$). $P < 0.01$ as compared with basal value.

From table 1 it is obvious that in 60-and 120-day old animals hydrocortisone hemisuccinate administered during pentobarbital anaesthesia strongly potentiates the hyperglycemic effect of the anaesthetic drug (with 17.1 and 30.8%, $P < 0.05$), while in immature (30-day old) and in old animals (more than 1 year) it fails to affect the hyperglycemia. We consider that the above differences are due to a great extent to the age-dependent rapid antiinsulinic effect of this glucocorticoid, dependence previously proved by us but *in vivo* [13] and *in vitro* [14].

CONCLUSIONS

1. During short-term sodium pentobarbital anaesthesia the fasting glycemia in intact rats of various ages substantially increases.
2. Adrenals in white rats play a major role in the mechanism of hyperglycemic action of sodium pentobarbital.
3. Hydrocortisone hemisuccinate may potentiate the pentobarbital induced hyperglycemia, depending on the age of white rats.

REFERENCES

1. Bailley C. J., Atkins T. W., Matty A. J., 1975, Endocrinologia experimentalis, **9**, 177—184.
2. Bouman P. R., Dermer W., 1960, Acta endocr. (Kbh), **35**, 541—550.
3. Brunner E. A., Haugaard N., 1965, J. Pharmacol. exp. Ther., **150**, 99—104.
4. Davidson M. B., 1971, Horm. Metab. Res., **3**, 243—247.
5. Green M. A., Allen C. F., 1975, Neuroendocrinology, **17**, 258—264.
6. Howard N. J., Martin J. M., 1972, Endocrinology, **91**, 1513—1515.
7. Jolin T., Montes A., 1973, Hormone Res., **4**, 153—156.
8. Krebs H. A., Bennett D. A. H., Degasquet P., Gascyone T., Yoshida T., 1963, Biochem. J., **86**, *1*, 22—27.
9. Lecklercq-Meyer V., Malaisse W. J., 1975, Diab. Metab., **1**, *3*, 119.
10. Madar J., 1966, Doctoral Thesis, Univ. Cluj, Romania,.
11. Madar J., Pora E. A., 1970, Ann. Endocrinol. (Paris), **31**, 1081—1086.
12. Madar J., Sildan N., Pora E. A., Arch. int. Physiol. Biochim., 1972, **80**, 367—371.
13. Madar J. Sildan N., Pora E. A., 1975, Ann. Endocrinol. (Paris), **35**, 25—30.
14. Madar J., Sildan N., Pora E. A., 1975, Rev. Roum. Biol. Ser. Biol. Anim., **2**, 131—134.
15. Randle P. J., 1964, Ciba Found. Colloq. Endocr., **15**, 137.
16. Roberts P., Turnbull M. J., Winterburn A., 1970, Europ. J. Pharmacol., **12**, 375—377.
17. Saiton Y., Ui M., 1975, Biochem. Biophys. Acta, **405**, *1*, 7.
18. Sharpless S., 1970, In: *The pharmacological basis of therapeutics*, 4th ed., (Eds. L.S Goodman and A. Gilman), Macmillan Co., London, pp. 98—120.
19. Spitzer J. J., McElroy W. T., 1961, Amer. J. Physiol., **201**, 815.
20. Turner D. M., 1975, Res. Comm. Chem. Pathol. Pharmacol., **12**, *4*, 156.

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INTERACTION BETWEEN PROCAINE AND OUABAIN AT THE MUSCLE FIBRE MEMBRANE LEVEL

BY

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Experiments were performed on frog sartorius muscle fibres by the intracellular glass microelectrodes technique, at pH= 7.2 and 6.0. Ouabain (5×10^{-6} M) leads not only to an inhibition of the ($Na^+ - K^+$)-pump but to a weak membrane depolarization in an alkaline medium and a hyperpolarization in an acid medium as well. The pump inhibitor hinders binding of procaine to the membrane and decreases its specific bioelectric effects at pH = 7.2 as well as at pH = 6.0, but the anesthetic influences its effects too. The combined action of these agents depends on the state the membrane is in: at membranes with a normal resting structure the procaine effect is more evident whereas at the initially procaine modified membrane predominates the ouabain effect.

Investigations concerning the interaction of local anesthetics with the ($Na^+ - K^+$)-pump [4], [5], [20] showed that in clinically used concentrations, these agents do not directly influence active transport but may inhibit it by intervening in the passive cation fluxes. But the authors underlined the complexity of the problem and the fact that many aspects are unsolved.

In order to deepen the understanding of these phenomena, in the present paper the interference between the effects of a pump inhibition, ouabain, and those of a local anesthetic, procaine, upon the electrical charge of the striated muscle fibre membrane was followed up.

MATERIAL AND METHODS

Investigations were carried out on frog (*Rana ridibunda*, Pall.) sartorius muscle fibres, by the technique of intracellular glass microelectrodes. Each experiment was conducted on five muscles, at room temperature. Ringer solutions with bicarbonate buffer at pH = 7.2 and with phosphate buffer at pH = 6.0 were used. 5×10^{-6} M ouabain (Nutritional Biochemicals Corp., Cleveland, Ohio) solutions and 2.5 mM and 1 mM procaine (Procainhydrochloride) (Merck) solutions were prepared by adding the substances to Ringer. The statistical significance was calculated according to Student's *t* test.

RESULTS

The average value of the resting potential registered in our experiments was between 92.08 mV and 94.88 mV (SE about 0.50 mV) (Figs. 1 and 2 : RP).

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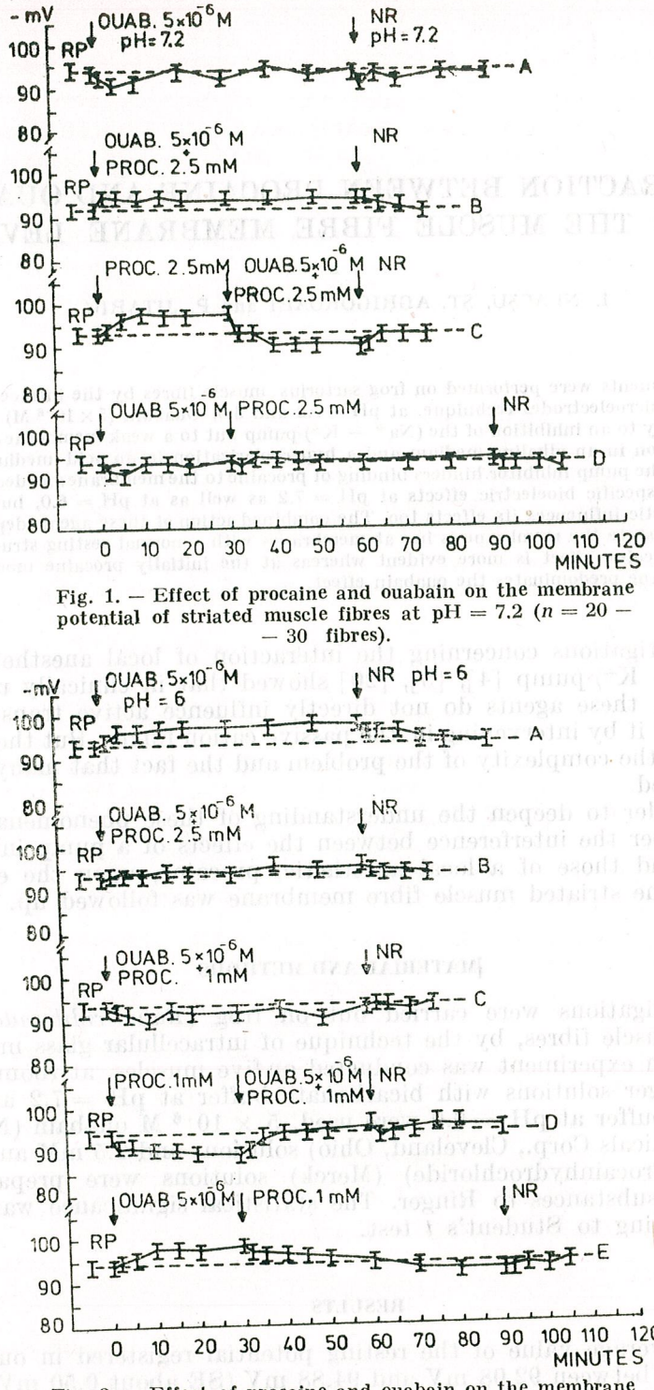

Fig. 1. — Effect of procaine and ouabain on the membrane potential of striated muscle fibres at pH = 7.2 ($n = 20$ — — 30 fibres).

Fig. 2. — Effect of procaine and ouabain on the membrane potential of striated muscle fibres at pH = 6.0 ($n = 20$ — — 30 fibres).

In control experiments the effect of 5×10^{-6} M ouabain on the membrane resting potential at pH = 7.2 and 6.0 was established. At pH = 7.2, in an ouabain-Ringer solution (Fig. 1-A) a weak depolarization during the first minutes (3.78 mV amplitude) can be seen after which the membrane potential oscillates around the resting value, not being modified even after washing of the fibres with normal Ringer (NR). Such an effect of ouabain was also reported by other authors [10], [14], [15]. On the other hand, at pH = 6.0 a persistent membrane hyperpolarization (4.09 mV average amplitude), reversible in normal Ringer, was found (Fig. 2-A).

The interference between the effects of ouabain and procaine was followed up at the same pH values, because the effects of the two agents proved to be pH dependent [3].

In a first series of experiments at pH = 7.2 in Ringer containing 5×10^{-6} M ouabain and 2.5 mM procaine (Fig. 1-B) a constant hyperpolarization was found, but much weaker (1.93 mV amplitude) than when procaine was given alone (4.69 mV amplitude) [3]. At a simultaneous action of the two agents on the membrane already hyperpolarized by 2.5 mM procaine (Fig. 1-C), a rapid return to resting membrane potential value takes place, followed by slow and feeble depolarization (3.43 mV maximal amplitude), reversible in normal Ringer. When procaine is given after the installation of the ouabain action (Fig. 1-D) the hyperpolarization characteristic of the anesthetic does not appear any more.

At pH = 6.0, two procaine concentrations with different effects on the membrane were used : 2.5 mM (hyperpolarization) and 1 mM (depolarization) [3]. In Ringer with 5×10^{-6} M ouabain and 2.5 mM procaine (Fig. 2-B) a weak hyperpolarization is evident (1.30 mV amplitude) more reduced than the one given by ouabain or procaine only [3]. In medium with 5×10^{-6} M ouabain and 1 mM procaine (Fig. 2-C) a depolarization (about 3 mV amplitude) takes place, but much more reduced than the one given by procaine alone [3]. If ouabain and 1 mM procaine are acting after the membrane is already depolarized by procaine (Fig. 2-D), the ouabain effect is stronger, leading to a rapid resting potential rebuilding, but without the occurrence of the hyperpolarization characteristic of the pump inhibitor. When the fibres are treated with 1 mM procaine after the membrane hyperpolarization by ouabain (Fig. 2-E), the potential falls gradually to its resting value, followed by a feeble depolarization (1.52 mV maximal amplitude).

DISCUSSIONS

The electrogenic role of the (Na^+— K^+)-pump was extensively studied [1], [8], [11], [12], [13], [16], [17], but the correlation between the inhibition of the pump by cardiac glycosides and the electrical charge of the membrane has been less investigated [6], [7], [9], [14], [15], [18]. On the clarification of this problem depends the better understanding of the bioelectrogenesis in general as well as of the bioelectric effects of many agents, among others of local anesthetics too, which are explained either on the basis of an interplay with active transport, or by influencing passive diffusion.

Our experiments show that ouabain, besides a blocking action of the pump, exhibits bioelectrical effects of its own. Thus, at pH = 7.2, 5×10^{-6} M ouabain has a weak and transient depolarizing effect (Fig. 1-A). Such a phenomenon was reported by other workers too [10], [14], [15], [22], and it was shown that it is not present at higher concentrations (10^{-3}M). On the other hand, it was found that at smaller concentrations (10^{-8}M — 10^{-4}M), ouabain acts on the saturable component of the pump [6], [14]. Although it seems that a direct connection between the effect of pump blockage and depolarization due to ouabain exists, some authors deny this fact [21]. Because the weak depolarization by ouabain does not reflect accurately the extent of active transport reduction [17], we consider it to represent a secondary effect of the inhibition of the pump.

The experiments at pH = 6.0 revealed different aspects (Fig. 2-A). It has been shown that this pH does not modify the membrane potential in normal Ringer [2], [19], although it reduces the pump activity [17] and at the same time blocks the binding of ouabain to the membrane [6]. Under these conditions the presence of the inhibitor in the medium should not lead to a membrane potential modification. But the constant hyperpolarization obtained shows that ouabain at pH = 6.0 is bound in the membrane. On the other hand, the bioelectrical effect different from the one obtained at alkaline pH could be explained by different aspects of the active transport at this pH [17].

It was shown that at blocking concentrations, the local anesthetics do not affect directly the active transport [4], [5], [20]. Our data show that at such concentrations, at pH = 7.2, the simultaneous action of procaine and ouabain (Fig. 1-B) leads to a persistent and reversible membrane hyperpolarization, but to a much lesser degree than that specific to procaine [3]. This pronounced reduction cannot be explained by a simple counterbalance of the effects of the two drugs. In this case, as well as in the case of applying procaine after the installation of the effect of ouabain (Fig. 1-D), it may be admitted that the glycozide, at the same time with blocking the pump, inhibits also the binding of the anesthetic in the membrane , and reduces its efficiency in inducing the specific hyperpolarization, which shows that a correlation between the activity of the pump and the action of local anesthetics does exist.

When ouabain is added to the medium after the hyperpolarization by 2.5 mM procaine (Fig. 1-C) takes place, the phenomena are different. In this case ouabain removes the anesthetic from the membrane, reducing completely its specific effect. The difference as compared to the situation when the two agents act from the beginning together could reflect the fact that in this case the interaction between them takes place at the level of the micellar structure characteristic of the procaine hyperpolarization membrane [3], and not at the level of the resting structure. After the hyperpolarization is abolished, only the ouabain specific depolarization remains expressed, even with a tendency of slight accentuation.

Similar conclusions may also be drawn from the experiments at pH = 6.0. In such conditions procaine leads either to a hyperpolarization (2.5 mM), or to a depolarization (1 mM) [3], whereas ouabain has a membrane hyperpolarizing effect. When procaine and ouabain act together from the beginning, a reduction of both effects of the anesthetic can be

seen. This may express either a reciprocal reduction of the two agents effects, at 2.5 mM procaine (Fig. 2-B), or a summing-up of two different effects with different amplitudes, at 1 mM concentration (Fig. 2-C). If ouabain acts on the already 1 mM procaine depolarized-membrane (Fig. 2-D), the pump inhibitor will abolish rapidly the effect of the anesthetic, but will not lead to its specific hyperpolarization any more. Procaine also abolishes the effect of ouabain but much more slowly, as can be seen from the experiments in which the membrane already hyperpolarized by the inhibitor is treated with anesthetic (Fig. 2-E).

An antagonism between the action of the two drugs may be seen, both in alkaline medium and in acid medium. Ouabain reduces the efficiency of procaine, but the anesthetic in general reduces the bioelectric effect of the pump inhibitor too. The combined action of these agents depends on the membrane state, the pH determining only the nature of their specific effect. When they act on a membrane with a structure characteristic of the normal resting state, the specific effect of procaine is more evident, whereas when they act on the membrane already modified by the anesthetic, the specific effect of ouabain is more evident. The efficiency of ouabain is increased however, when it acts on the membrane hyperpolarized by 2.5 mM procaine and is decreased when it acts on the membrane depolarized by 1 mM procaine.

Besides this, our data support the idea of a relatively reduced electrogenic role played by the (Na^+— K^+)-pump. The study of such phenomena is complicated, however, by the fact that the pump inhibitor shows some bioelectrical effects of its own.

REFERENCES

1. Adrian R. H., Slayman C. L., 1966, J. Physiol. (London), **184**, 970.
2. Agrigoroaei Șt., Neacșu I., 1976, *A II-a Conf. Biol. Cel.*, București, 12—14 febr., C (14), p. 39.
3. Agrigoroaei Șt., Neacșu I., 1977, Rev. Roum. Biol. — Biol. Anim., **22**, *2*, 155.
4. Andersen N. B., 1968, J. Pharmacol. Exp. Therap., **163**, 393.
5. Andersen N. B., Amaranath L., 1973, Anesthesiology, **39**, *2*, 126.
6. Baker P. F., Willis J. S., 1972, J. Physiol. (London), **224**, 463.
7. Carpenter D. C., 1973, Science, **179**, **4080**, 1336.
8. Carpenter D. C., Alving B. C., 1968, J. Gen. Physiol., **52**, p. I, 1.
9. Chipperfield A. R., Whittam R., 1973, Nature, **5392**, 62.
10. Crăciun Margareta, Agrigoroaei Șt., 1978, Rev. Roum. Biol. — Biol. Anim., **23**, *2*, 143.
11. Cross S. B., Keynes R. D., Rybova R., 1965, J. Physiol. (London), **181**, 865.
12. De Weer P., 1975, *Physiology*, Series One, Vol. 3, *Neurophysiology*, Ed. by C. C. Hunt, Butterworths London, Univ. Park Press, Baltimore, p. 232.
13. Glynn I. M., Karlish S. J. D., 1975, Ann. Rev. Physiol., **37**, 13.
14. Grossie J., 1976, J. Cell. Physiol., **88**, *1*, 117.
15. Johnson J. A., 1965, Amer. J. Physiol., **187**, *2*, 328.
16. Kernan R. P., 1963, Nature, **193**, 986.
17. Keynes R. D., 1965, J. Physiol. (London), **178**, 505.
18. Keynes R. D., Steinhardt R. A., 1968. J. Physiol. (London), **198**, 581.
19. Narahashi T., Frazier D. T., 1971, Neurosci. Res., **4**, 66.
20. Orloff J., Handler J. S., 1964, Amer. J. Med., **36**, 686.
21. Shanes A. M., 1968, Pharmacol. Rev., **10**, 58.
22. Zierler K. L., 1959, Amer. J. Physiol., **198**, *4*, 1066.

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PHYSICO-CHEMICAL BASIS OF THE TETRAALKYLAMMONIUM ION INTERACTION WITH THE CELL MEMBRANE

BY

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Although the tetraalkylammonium ions are hydrosoluble quaternary compounds, they are able to act on the excitable fiber membranes due to their "structure making" properties. On the other hand, according to hydrophilia/hydrophobia ratio which is characteristic of their structure, they can be differentiated in stabilizing and depolarizing ions. For the stabilizing ions the hydrophobia does not exceed hydrophilia. If polar groups were introduced in their structure, this gained "structure breaking" properties and the stabilizing action was greatly reduced. The depolarizing ions have prevalently hydrophobic properties.

As we have previously shown [4], a number of inferior ions in tetraalkylammonium series (TMFA, TEA, TPA) have stabilizing effects on the muscle fiber membrane, while TBA ion produces its persistent depolarization [3].

A similar differentiation of the membrane effects has also been reported by other authors [29] after internal perfusion of squid giant axon with sodium or tetraalkylammonium ions while the axon was maintained in an external medium containing a single electrolytic species of a divalet cation ($CaCl_2$). The axon membrane responded to an excitation by action potentials after perfusion with Na, TMA, TEA and TPA, but not with TBA.

Investigations on artificial lipidic membranes [13], [24] have shown that the TBA transport number (across these membranes) was 30 — 80 times higher than that of TMA and TEA.

As the stabilizing effect at the level of ganglionic postsynaptic membrane is concerned, it was shown [23], [27] that this is increasing with the number of ethyl groups which replace methyl groups in TMA structure. Moreover, the specific ganglioplegic action increased if one of the ethyl radicals from TEA structure was replaced by a propyl, butyl or phenyl group. However, when two ethyl groups are replaced by bigger alkyl radicals the optimal activity is observed for dipropyl compound, the dibutyl derivative being already less active than TEA.

Radical branching increases the ganglioplegic action, the isopropyl congener being 2.5 times more active than TEA and diisopropyl derivative about 12 times more active.

Introduction of a hydroxyl group in TEA structure giving hydroxyethyltriethylammonium decreases to half its ganglioplegic activity. If the hydroxyl group was acetylated to form the triethyl, analogue to acetylcholine, the stabilizing blocking action was maintained [12].

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The action of tetraalkylammonium ions at the level of motor end-plate is more complex [9], [18]. Yet, a stabilizing effect has been observed which increased with the number of methyl radicals in TMA replaced by ethyl or propyl radicals. Butyl radicals do not favor this action [12]. In general, in the case of compounds with four or more carbon atoms in radicals (butyl, hexyl, octyl) a depolarization rather than a stabilization of the membrane occurred.

No special problem arised from the fact that these ions act on excitable membranes along the fibers, although it is well known that in general quaternary ammonium derivatives have no effects outside junction zones, since they are insoluble in lipids [28]. The solubility of tetraalkylammonium salts in water does not prevent such effects.

An explanation of this uncommon behavior seemed of interest to us, as we claimed [2] that membrane components involved in these interactions were the phospholipids and not the proteins. An assembly of data on the physico-chemical properties of alkylammonium salts and their derivatives, published lately, seemed to be relevant in this respect.

Thus, it was shown that the solubility of these compounds in water decreases with the increase of the cation organic character (radical elongation). In general, it is smaller for tetrasubstituted ions than for di- or monosubstituted ones.

In the determination of these salts solubility other factors are also involved such as symmetry or asymmetry of the structure (identical or different radicals) or the nature of anion, but in general, the hydrophobic character of the organic radicals plays a prevailing role in decreasing the solubility in water [11].

On this basis, certain ratios between the structure hydrophilia and hydrophobia are established, ratios which generally show an increased lipophilia of ammonium quaternary salts compared to amine salts and in the former case form those with small radicals toward those with bigger radicals.

Tetrabutylammonium solubility in benzene is already a little higher than in water [16].

Some other special properties of tetraalkylammonium ions have been observed, which are not encountered for the solutions of other substances. Differently from most electrolytes, the presence of which in the medium determine an acceleration of the reactions (saline kinetic effect) the tetraalkylammonium salts lead to a decrease of the reaction rates [14], [17], [30].

Their aqueous solutions have an abnormal behaviour as far as thermodynamic properties are concerned, exhibiting uncommonly high negative values of the entropy [25].

Introduction of hydroxyl groups in the alykl catena of tetraalkylammonium ion modifies its transport properties [8] and the coefficient of activity, [31], the ion thus behaving more similar to alkaline cations. Another effect of introduction hydroxyl groups in alkyl catena is the net increase of solubility in water. Substitution of a methyl by a hydroxyl in the tetrapropylammonium ion, although the ionic radius is unchanged, leads to an increase in conductibility and a decrease of solution viscosity. The coefficient of viscosity, which readily increases with the rise of temperature for the solutions of tetraalkylammonium salts, remains constant for the solutions of tetrahydroxyalkylammonium salts [11].

In order to explain all these unusual properties, the model proposed by H. S. Frank and W. Y. Wen [10] was adopted, later supported by numerous subsequent proofs [5], [6], [19], [22].

According to this theory, the molecules or ions with hydrophobic radicals in an aqueous solution exhibit "structure making" properties, that is they induce a structuring of water near to aliphatic catenas, by a sensible increase in the number of water molecules bound by hydrogen bonds. The so-called "clusters" of solvent molecules are formed, which lead to a decrease of the free water-fraction in the solution. A smaller contact surface of the organic cation with water occurs and its greater ease to insert in existent "cavities" between "clusters", that is in zones where the hydrogen bond forces are weaker.

This water structure making explains the unusual high negative values of solution entropy. In their turn, the increase of conductibility, decrease in viscosity and the increase of solubility in water of tetraalkylammonium salts when hydroxyl groups are introduced in their structure are explained by the "structure breaking" effect of these polar groups on surrounding water.

The hydrophobic nature of alkyl catene of tetraalkylammonium ion is exceedingly compensated by introduction of a hydroxyl polar group, thus eliminating the structuring of surrounding water.

The increase of cation lipophilia together with the increase of organic radical size is well explained by this model, as well as the fact that they are found in a unhydrated form in the aqueous solution [25], [26] (also due to their great ionic radius). Their hydration occurs only when polar groups are introduced in the structure of organic radicals.

Of great importance is also the ability of tetraalkylammonium ions to form with anions, in certain conditions, ionic pairs, which allows these strong electrolytes to behave in solution like other nondissociated substances [7]. The decrease of the cation organic character as well as its hydration after introduction of polar groups in the structure prevents the salt to form an association of ionic pair type.

Based on the facts mentioned above, we consider that not only the different behaviour of tetraalkylammonium ions could be explained as compared to other quaternary ammonium ions with more complex structure in relation to excitable fiber membranes, but also the differentiation of effects inside this series of organic cations.

Although they are in fact hydrosoluble, like the quaternary ammonium ions with complex structure, their hydrosolubility is not typical. They do not hydrate and induce at the same time water structuring, thus succeeding easily to insert between solvent "clusters". By exhibiting a high degree of independence related to the aqueous medium, they are able to get close to the membrane phospholipidic structures. A certain ratio between the structure hydrophilia and hydrophobia, in the conditions of its symmetry, allows it to get from the extracellular aqueous phase in the membrane lipidic phase and to interact with its structural components.

Since the stabilizing action, at the level of the membrane, of the inferior members of the series is more pronounced as the organic radicals

conferring lipophilia to the structure are longer or more branched [4]' [12], it is obvious that the final characteristic effect depends on their degree of lipophilia.

The adsorption of these organic ions in the protection sphere of phospholipidic micellae is stronger than that of sodium ions, to this contributing not only the electric interactions but also those based on hydrophobic forces.

However, it is clear enough that a stabilizing effect is only possible as long as the hydrophilia-hydrophobia ratio is shifted toward prevailing hydrophobia. It is significant, from this point of view, that the increase of stabilizing action in the series TMFA, TEA, TPA is made by continuously smaller rates [1]. On the other hand, the TBA ion, the solubility of which in organic solvent already exceedes that in water [16], achieves a new type of effect, that is a depolarization of the membrane, which is more pronounced for the ions with more carbon atoms in the radicals.

In relation to the marked loss of stabilizing properties of hydroxylated derivatives of tetraethyl and tetrapropyl ammonium ions [12] or the reduced stabilizing ability of trimethylhydroxyethylammonium and dimethyldihydroxyethylammonium ions [20], [21], it is obvious that this loss is derived from the "structure breaking" properties, induced by the presence of polar groups in the structure. The hydrophilia of these compounds increases so much that they are no longer able to pass easily from the aqueous phase (extracellular medium) in the lipidic phase (membrane). Thus their behaviour is similar to that of hydrosoluble complex quaternary ammonium ions.

The tetraalkyllammonium ions with four or more carbon atoms in the organic radicals preserve their solubility in water although lipophilia of their structure becomes higher than their hydrophilia.

Their "structure making" properties are not lost, but the effect on membrane lipidic structures is changed from a stabilizing to a transitional one, which determines the membrane depolarization. The high lipophilia allows their insertion between the molecules of phospholipidic micellae and the substitution of their characteristic structuring ion.

Therefore, these ions behave differently as compared with the hydrosoluble complex quaternary ammonium ions but very similarly to their congeners with lipophilic properties induced by chemical modification [15], [32].

In conclusion, it can be stated that :

1. The ability of tetraalkylammonium ions to act on the cell membranes is mainly based on their special "strucure making" properties.

2. The differentiation of the effects, stabilizing and depolarizing, in the series is based on the ion hydrophobia and hydrophilia ratio. When hydrophobia does not exceed hydrophilia, the characteristic effect is stabilizing. When the hydrophobic properties prevail, the typical effect is depolarizing.

3. The stabilizing ions greatly or totally lose this capacity when polar groups are in their structure, which induce "structure breaking" properties, these ions becoming strongly hydrosoluble.

4. To explain the specific effects of tetraalkylammonium ions one should consider, on the one hand, their different behaviour in the extracellular medium (especially toward the solvent : water), and on the other hand, their behaviour toward phospholipidic structures from the membrane lipidic leaflet.

REFERENCES

1. Agrigoroaei Șt., 1974, *Cercetări asupra mecanismelor biofizice ale proceselor de excitație și, permeație la nivelul organizării moleculare a membranelor fibrelor excitabile* (Thesis), Iași.
2. Agrigoroaei Șt., 1976, Rev. Roum. Biol. — Biol. Anim., **21**, 137.
4. Agrigoroaei G., Agrigoroaei Șt., 1978, Rev. Roum. Biol — Biol. Anim., **23**, 149.
4. Agrigoroaei Șt., Agrigoroaei G., Patapie-Raicu Fl., 1979, Rev. Roum. Biol.—Biol. Anim., **24**, 35.
5. Clifford J., Pethica B. A., 1965, Trans. Faraday Soc., **61**, 182.
6. Darnell A. J., Greyson J., 1968, J. Phys. Chem. **72**, 3021.
7. Diamond R. M., 1963, J. Phys. Chem. **67**, 2513.
8. Evans D. F., Cunningham G. P., Kay R. L., 1966, J. Phys. Chem., **70**, 2974.
9. Frank G. B., 1961, Brit. J. Pharmacol., **17**, 59.
10. Frank H. S., Wen W. Y., 1957, Discussion Faraday Soc., **24**, 133.
11. Giacomelli A., Manicagli R., 1969, Ann. Chim. (Roma), **59**, *10*, 860.
12. Goodman L. S., Gilman A., 1955, *The Pharmacological Basis of Therapeutics*, Macmillan Comp., New York.
13. Goudeau H., 1967, J. Physiol., (Paris), **59**, 417.
14. Healy R. M., Kilpatrick L. M., 1955, J. Am. Chem. Soc., **77**, 5258.
15. Hinterbuchner L. P., Wilson I. B., Schoffeniels E., 1958, Federation Proc., **17**, 71.
16. Highes D., Ingold C. K., Patai S., Pocker Y., 1957, J. Chem. Soc. (London), 1207.
17. Indelli A., 1961, J. Phys. Chem., **65**, 972.
18. Koketsu K., 1958, Am. J. Physiol., **193**, 213.
19. Lucas M., Marciaqo-Rousselot M. M., 1972, C. R. Acad. Sc. (Paris), **274**, (Série C), 312.
20. Lundberg A., 1951, Acta Physiol. Scand., **22**, 365.
21. Lundberg A., Oscarsson O., 1953, Acta Physiol. Scand., **30**, 99.
22. Miller K. W., Hildebrand J. H., 1968, J. Am. Chem. Soc., **90**, 3001.
23. Moe G. K., Freyburger W. A., 1950, Pharmacol. Rev., **2**, 61.
24. Monnier A. M., 1968, J. Gen. Physiol., **51**, 26 s.
25. Nemethy H. A., Scheraga H. A., 1962, J. Chem. Phys., **36**, 3382.
26. Nightingale E. R., 1959, J. Phys. Chem., **63**, 1381.
27. Paton W. D. M., Perry W. L. M., 1953, J. Physiol. (London), **119**, 43.
28. Rothenberg A. M., Sprinson D. B., Nachmansohn D., 1948, J. Neurophysiol., **11**, 111.
29. Tasaki I., Lerman L., Watanabe A., 1969, Am. J. Physiol. **216**, 130.
30. Wazer J. R., van Griffith E. J., McCullough J. F. 1955, J. Am. Chem. Soc., **77**, 287.
31. Wen W. Y., Saito S., 1965, J. Phys. Chem., **69**, 3569.
32. Wilson I. B., 1958. Biochim. Biophys. Acta, **27**, 196.

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A NEW POINT OF VIEW ON THE MECHANISM OF ACTION POTENTIAL

BY

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On the basis of "2—M.S.I." concept, the action potential (AP) is explained as a result of direct and reverse phase transitions of micellar structures of the two membrane layers. These are produced by ion movements and are accompanied by changes in electrical charge and specific permeability of the layers. The ascendent phase of AP is due to the action of both external Na^+ on the internal layer (direct depolarization) and internal K^+ on the external layer (reverse polarization).

The descedent phase results from the action of internal K^+ on the external layer (reverse depolarization) and that of external Ca^{2+} on the external layer (direct repolarization).

The development of action potential (AP) cannot be fully explained only either on the basis of an exchange of electrical charges between the two sides of the membrane [13] or on some phase transition phenomena occurring in the membrane external layer [20].

Na^+ and K^+ fluxes specific to the active state of the membrane, as shown by voltage-clamp experiments, are neither netly separated during the two phases, as they are considered by the ionic theory, nor equally high during the action potential, as presented by the theory of the two stable states.

The former theory does not suggest any mechanism for the ionic permeability changes, whereas the latter gives a very brief explanation for it.

A re-evaluation of the experimental results regarding the variations of both Na^+ and K^+ conductance during the excitation, considering our concept on the membrane structure and properties [3], led us to a point of view which gives a more satisfactory explanation for the intimate mechanisms of action potential development.

In this respect, some aspects regarding the specific effects of Na and K ions on the excitable fiber membrane should be recalled and specified.

It is known that an increased Na^+ concentration in the intracellular medium (by perfusion) results in a depolarization of the membrane [7] which is unfavorable for the maintenance of its normal excitability [17]. Extracellular sodium plays an important role in the maintenance of excitability, and the mechanism of its action has been previously presented [5].

On the other hand, at high internal concentrations K^+ does not modify the value of resting potential [7], being an internal cation favorable to the maintenance of excitability [17]. However, at high external concentrations, K^+ depolarizes the membrane [1] being the most unfavorable external cation it respect to fiber excitability [17].

If a depolarization of a fiber is simultaneously produced, on the one hand by internal perfusion with Na^+ in high concentration and, on the other hand, by keeping the fiber in an external medium with high

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K^+ concentration, the membrane potential has a normal value, but with opposite polarity [10].

We interpreted these phenomena as derived from the ability of each of the membrane layers to establish specific interactions with the adjacent phase based on their characteristic ion exchange properties [2].

It is evident that Na^+ effect on the membrane internal layer, when its concentration is increasing, is identical, regardless if its increase is produced by Na^+ coming from inside (as in passive depolarization) or outside (as in active depolarization) of the membrane.

There is a general agreement regarding the depolarizing effect of K^+ in high external concentration. However, the action of this ion at the level of the external layer when its concentration is increasing, from the internal medium is differently interpreted. In view of the ionic theory it is considered that the K^+ efflux in the descendent phase of action potential produces a depolarization of the membrane, whereas the theory of the two stable states explains the active depolarization itself (the ascendent phase of AP) as determined by K^+ efflux from the fiber.

We have already shown in a previous paper [2] that there is no proper proof to ascribe K^+ different actions at the level of external layer related to ist increasing from the internal or external medium. The only effect which can be admitted is that observed in passive depolarization, but the electrical charge of the membrane in resting or active state is differently influenced [4].

Another important aspect which should be taken into account is that of ion flux amplitude during the action potential. It was shown that for the total variation of the membrane electrical charge a movement of an extremely small amount of ions is needed [12]. Moreover, the net Na^+ and K^+ fluxes do not modify significantly the concentrations of these ions in the intra- and extracellular medium, respectively [14], where the recording electrodes are placed, but carry an amount of charge about three times higher than that needed to produce the electrical potential variation.

Therefore, it should be admitted that the potential variations are not a result of a simple transfer of charge from one side of the membrane to the other, and that the structure, properties and electrical charge of each layer of the membrane are greatly modified by the action of ion coming from the opposite extramembranary phase.

Thus, in principle, an inward penetration of Na^+ ions to the level of internal layer would determine a modification of its anionic exchange micellar structures in cationic exchange structures, which results in a reverse of the resting electrical charge of this layer. If this phenomenon was not accompanied by other changes at the level of the membrane as an assembly, one would observe a simple depolarization (of passive type) due to the fact that the electrical polarities of the two layers in resting state are identical.

Similarly, if the possibility of a simple outward passage of internal K^+ ions was admitted, they would produce a transformation of cationic exchange micellar structures from the external layer in anionic exchange structures with a reversal of the electrical charge specific to its resting state.

For the reasons mentioned above, once again, the resulting general effect would be a depolarization of the membrane.

However, in reality the development of the events during action potential is much more complex.

The membrane depolarization to the threshold value is actually produced with no change in ionic permeability [5] and in disagreement with the affirmations of the ionic [11] and the two stable states [19] theories, the voltage-clamp experimental data also support this point of view (Fig. 1, RP-THR).

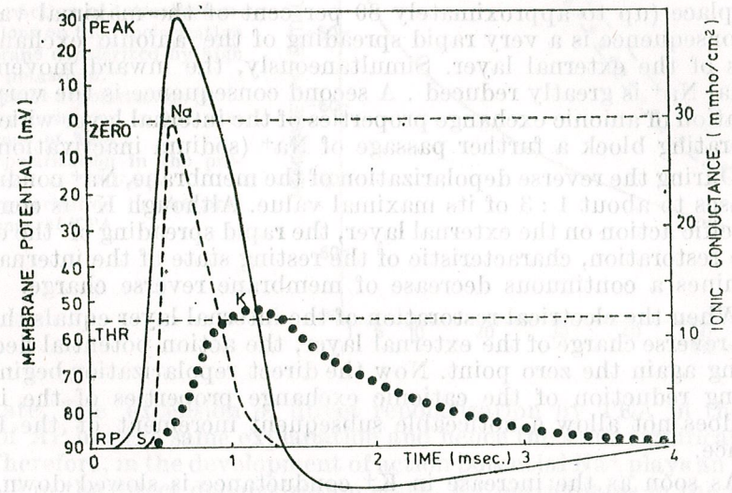

Fig. 1. — Action potential and ionic conductance variations.
—— Membrane potential; ——— Sodium conductance; ● ● ● Potassium conductance.

Once the threshold is exceeded, sodium is driven from the external toward the internal layer leading, by its specific action on the latter, to the direct active depolarization of the membrane (direct depolarization being the falling of the potential from the resting value to zero).

The rapid transformation of the internal layer in a cationic exchange layer allows a massive inward Na^+ penetration, its conductance presenting a very steep ascendent curve (Fig. 1,S-Na). However, the increase of Na^+ conductance is suddenly stopped when the membrane potential value is about zero and immediately thereafter the conductance decreases.

The ascendent phase of action potential is further continued, occurring a reverse depolarization of the membrane (Fig. 1, ZERO-PEAK).

For its explanation one should take into account that the internal layer, which became cationic exchanger, allows intracellular K^+ efflux. However, the increase of sodium conductance is accompanied from the begining by a slight K^+ efflux. But during the reverse polarization of the membrane, K^+ conductance increases markedly. This outward K^+ movement (toward the external layer) results in a limited reversal of its electric charge.

Since the electric polarity of the internal layer has already been reversed, the membrane as an assembly is gradually reverse polarized to the peak value.

As soon as the K^+ reduces the spreading of cationic exchange areas in the external layer, the increase of Na^+ influx is stopped and in a very short time even starts to decrease. This results in a restoration of internal layer anionic exchange properties, which inhibits the reverse polarization of the membrane. From this moment starts the descendent phase of action potential which also occurs in two distinct stages. During the reverse depolarization (Fig. 1, PEAK-ZERO) a marked increment in K^+ conductance takes place (up to approximately 80 per cent of the maximal value). A first consequence is a very rapid spreading of the anionic exchange properties of the external layer. Simultaneously, the inward movement of external Na^+ is greatly reduced . A second consequence is the very quick restoration of anionic exchange properties of the internal layer which while regenerating block a further passage of Na^+ (sodium inactivation).

During the reverse depolarization of the membrane, Na^+ conductance decreases to about 1 : 3 of its maximal value. Although K^+ is continuing its specific action on the external layer, the rapid spreading of the electric, charge restoration, characteristic of the resting state of the internal layer, determines a continuous decrease of membrane reverse charge.

When the electrical restoration of the internal layer equals the value of the reverse charge of the external layer, the action potential decreases, reaching again the zero point. Now the direct repolarization begins. Such a strong reduction of the cationic exchange properties of the internal layer does not allow a noticeable subsequent increment of the K^+ conductance.

As soon as the increase in K^+ conductance is slowed down, while simultaneously Na^+ conductance is rapidly decreasing, the phase transition effect of K^+ in the external layer ceases, taking place a reverse phase transition on account of Ca^{2+} rebinding in the structure.

This gradually leads to the restoration of cationic exchange properties and electric polarity specific to the resting state of the external layer. Now, the polarity of the two layers being the same, the membrane potential, as an assembly, regains normal polarity and rises up to the resting value.

The almost total inactivation of Na^+ brings about an inactivation of K^+.

Since, when the membrane potential reaches the resting value, the membrane is still slightly superionized, a final hyperpolarization appears which is gradually attenuated as the K^+ excess is removed from the membrane.

A proof showing that the direct repolarization stage is not due to K^+ action on the external layer but rather to Ca^{2+} is that of marked delay of this stage by TEA, effect described by several workers [8], [9], [15].

The phenomenon was interpreted as a result of K^+ conductance inhibition in this stage of the descending phase of action potential [6], [16], [18], since K ions were considered responsible for the active repolarization of the membrane.

However, our experimental data showed that TEA and other related ions (TMFA, TPA) strongly oppose to muscle fiber membrane repolarization, previously depolarized by high external K^+ (Fig. 2).

In fact, this means that these organic ions prevent the action of external Ca^{2+} on the depolarized membrane delaying its return to the resting state. The extension of direct repolarization by TEA in the final phase of AP has the same explanation and hence the same significance.

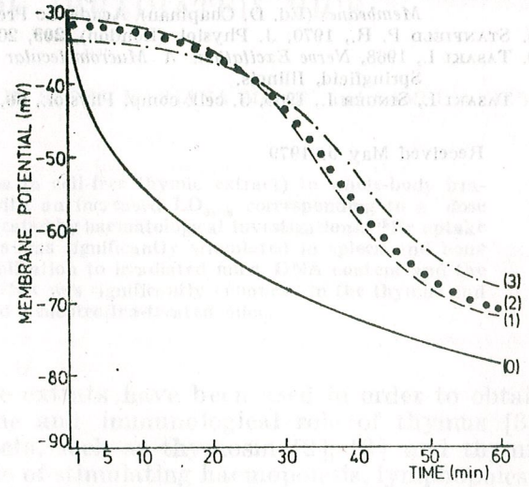

Fig. 2. — Action of tetraalkylammonium ions on the repolarization of membrane depolarized by high external potassium.
(0) — Normal repolarization;
(1) — Repolarization in the presence of TEA;
(2) — Repolarization in the presence of TMFA;
(3) — Repolarization in the presence of TPA.

Therefore, in the development of action potential Na^+ plays an important role in the direct depolarization stage, K^+ participates in the reverse polarization and repolarization, while Ca^{2+} acts in the direct repolarization stage.

The ionic permeability variations are based on direct and reverse phase transitions of the micellar structures characteristic of each layer. The automatic development of the excitation process is ensured by the feedback connections described.

REFERENCES

1. Adrian R. H., 1956, J. Physiol. (London), **133**, 631.
2. Agrigoroaei Şt., 1974, *Cercetări asupra mecanismelor biofizice ale proceselor de excitaţie şi permeaţie la nivelul organizării moleculare a membranei fibrelor excitabile*, (Thesis) Iaşi.
3. Agrigoroaei Şt., 1976, Rev. Roum. Biol. — Biol. Anim., **21**, 137.
4. Agrigoroaei Şt., 1976, *A II-a Conferinţă de Biologie Celulară*, Bucureşti, p. 36, C(8).
5. Agrigoroaei Şt., Agrigoroaei G., Patapie-Raicu Fl., 1979, Rev. Roum. Biol. — Biol. Anim., **24**, 35.
6. Armstrong C. M., 1966, J. Gen. Physiol., **50**, 491.
7. Baker P. F,. Hodgkin A. L., Shaw T. I., 1961, Nature (London), **190**, 885.
8. Burke W., Katz B., Machne X., , 1953, J. Physiol. (London), **122**, 588.
9. Hagiwara S., Watanabe A., 1955, J. Physiol. (London), **129**, 513.
10. Hodgkhin A. L., 1967, *The Conduction of the Nervous Impulse*, Liverpool University Press, Liverpool.

11. Hodgkin A. L., 1958, Proc. Roy. Soc. (London), B, **148**, 1.
12. Hodgkin A. L., Huxley A. F., 1952, J. Physiol. (London), **117**, 500.
13. Hodgkin A. L., Huxley A. F., Katz B., 1952, J. Physiol. (London), **116**, 424.
14. Hodgkin A. L., Keynes R. D., 1955, J. Physiol. (London), **128**, 28.
15. Lorente De No R., 1949, J. cell. comp. Physiol., **33**, Suppl., 1.
16. Schmidt H., Stämpfli R., 1966, Pflügers Arch. ges., Physiol., **282**, 311.
17. Singer I. Tasaki I. 1969, *Nerve excitability and membrane macromolecules*, in *Biological Membranes* (Ed. D. Chapman), Academic Press, London, New York.
18. Stanfield P. R., 1970, J. Physiol. (London), **209**, 209.
19. Tasaki I., 1968, *Nerve Excitation. A Macromolecular Approach*, Ch. C. Thomas Publisher, Springfield, Illinois.
20. Tasaki I., Singer I., 1965, J. cell. comp. Physiol., **66**, Suppl. 2, 137.

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THE EFFECT OF THYMIC HUMORAL FACTORS UPON REGENERATION OF HAEMOPOIETIC AND LYMPHATIC TISSUES OF IRRADIATED MICE

BY

Z. URAY, ELENA RĂDULESCU, D. SUCIU, MARIANA MANIU and CAMELIA BANU

Administration of Leucotrofina (a cell-free thymic extract) to whole-body irradiated mice was associated with an increased $LD_{50/30}$ corresponding to a dose reduction factor of 1.21. As indicated by haematological investigations, ^{59}Fe uptake and ESC number, haemopoiesis was significantly stimulated in spleen and bone marrow after Leucotrofina application to irradiated mice. DNA content and the uptake of ^{3}H-thymidine into DNA was significantly enhanced in the thymus and bone marrow of irradiated and Leucotrofina-treated mice.

Various thymic cell-free extrats have been used in order to obtain information on the endocrine and immunological role of thymus [3]. Some of these thymic extracts, such as thymosin [2], [3] and thymic fraction B [4] which were able of stimulating haemopoiesis, lymphopoiesis and of increasing immunological competence, were also effective radioprotective and therapeutic agents [4], [9] — [12]. The aim of the present study was to investigate the radio-protective and therapeutic effect of Leucotrofina, a cell-free thymic extract produced by Ellem (Milano, Italy). It was found that application of Leucotrofina before or after whole-body irradiation of A2G and DBA mice was correlated with a significant increase of the mean survival time and an enhanced recovery of haemopoietic and lymphopoietic activity.

MATERIALS AND METHODS

1. *Irradiation*. Groups of 20 male A2G or DBA mice weighing 20 to 23 g were whole-body gamma-irradiated by using a ^{60}Co therapeutic unit (Theratron 80) (FSD 80 cm, 174 R/min) or X irradiated with a therapeutic unit TUR II (180 kV, 10 mA, 1 Cu.FSD 80 cm, 30 R/min).

2. *Leucotrofina application*. Mice were i.p. injected with single or repeated doses of 5 units of Leucotrofina (Ellem, Milano, Italy) a calf thymus cell-free extract. One vial contains 50 units/2 ml of Leucotrofina corresponding to 500 mg of fresh gland.

3. *Survival experiments*. Groups of 20 mice received daily 5 units of Leucotrofina for the subsequent 6 days following gamma-irradiation with 500 to 900 rad. According to the same schedule the control groups received 0.2 ml of saline at 24 hours intervals.

4. *^{59}Fe uptake in erythrocytes.* Groups of 20 A2G mice were whole-body X-irradiated with 100 rad. Animals received 5 units of Leucotrofina at 60 minutes before or after the radiation exposure. At 24 hours after irradiation mice were i.p. injected with 0.2 μCi ^{59}Fe (Fe citrate). The uptake of ^{59}Fe by erythrocytes was measured at 2,4 and 7 days after labelling, as previously described [8].

5. *Uptake of ^{59}Fe in spleen and bone marrow.* Groups of 20 DBA mice received 5 units of Leucotrofina daily for the subsequent 6 days after whole-body X-irradiation with 400 or 500 rad. The control groups were injected at the same time with 0.2 ml of saline. At 9 days after irradiation animals were i.p. injected with 0.5 μCi ^{59}Fe. After 4 hours mice were killed and gamma activity of left femur and spleen was counted. The ^{59}Fe uptake was expressed as percentage of the activity applied.

The endogenous spleen colonies were counted according to Till and McCulloch [7].

6. *DNA content and ^{3}H-thymidine uptake.* Groups of 10 A2G mice were X-irradiated with 500 rad. The treated group received 5 units of Leucotrofina immediately and for the subsequent 6 days following the radiation exposure, at 24 hours intervals. At 90 minutes before killing mice were i.p. injected with 12 μCi of ^{3}H-thymidine (5 Ci/mM, Amersham, England). DNA content was determined in thymus, spleen and bone marrow by using the diphenylamine colour reaction, as previously described [6]. ^{3}H-DNA radioactivity was measured with the aid of a liquid scintillation spectrometer (Intertechnique ABAC SL_{40}), as previously indicated [5].

7. *Hæmatologic investigations.* Groups of 20 DBA mice were whole-body X-irradiated with 400 rad. Animals received 5 units of Leucotrofina daily for the subsequent 6 days after irradiation. According to the same schedule the control group received 0.2 ml saline. Blood and bone marrow were taken for quantitative and qualitative determinations on the 2-nd, 9th, and 16 th days from the irradiation. Routine haematological methods were used.

8. *Statistical evaluation.* The results were treated statistically by means of Student *t*-test.

RESULTS

The survival of control and Leucotrofina- treated A2G mice which were irradiated with 500 to 900 rad is shown in table 1.

From the $LD_{50/30}$ data a dose reduction factor of 1.21 was calculated.

The uptake of ^{59} Fe into the erythrocytes of A2G mice irradiated with 100 rad untreated and treated with Leucotrofina is shown in table 2.

Administration of Leucotrofina before (D) or after irradiation (E) significantly stimulates the recovery of erythropoiesis, increasing the radioiron utilization with 80 — 100%.

The uptake of ^{59}Fe into the spleen and bone marrow on the 9th day after irradiation in untreated and treated DBA mice is shown in table 3.

Table 1

Survival, mean survival time and $LD_{50/30}$ of A2G mice

	Mean survival time (h)	% Survival per 30 days	$LD_{50/30}$
Control groups			
500 rad	565	65	
600 rad	490	45	
700 rad	324	20	570 rad
800 rad	190	0	
Treated with Leucotrofina			
500 rad	698	95	
600 rad	601	75	
700 rad	567	60	690 rad
800 rad	301	20	
900 rad	88	0	

Table 2

Uptake of ^{59}Fe in erythrocytes of A2G mice at different time intervals after the whole-body X-irradiation with 100 rad

Group	%Uptake of ^{59}Fe		
	3 days	5 days	8 days
A. Unirradiated control	37.7 ± 3.2	39.3 ± 1.3	41.9 ± 1.9
B. Leucotrofina 5u. 60 min. before sham irrad.	36.3 ± 2.0	39.7 ± 1.5	42.4 ± 1.6
C. Irradiated control	10.5 ± 0.7	12.8 ± 1.4	15.1 ± 1.6
D. Leucotrofina 5 u. 60 min. before irrad.	23.7 ± 2.5[a]	25.1 ± 1.7[a]	28.7 ± 2.7[a]
E. Leucotrofina 5.u 60. min. after irrad.	21.7 ± 0.9[a]	24.6 ± 0.7[a]	25.9 ± 0.2[a]

[a] — Statistical significance $p < 0.01$

In both experimental groups, irradiated with 400 or 500 rad, the recovery of splenic and medullary erythropoiesis is significantly stimulated in Leucotrofina-treated mice.

9 days after the whole-body irradiation of DBA mice with 500 rad the number of endogenous spleen colonies was of 8.51 ± 0.57 in the control group and significantly increased to 15.30±0.75 in Leucotrofina-treated mice.

Table 3

Uptake of ^{59}Fe in spleen and bone marrow of DBA mice at 9 days after whole-body X-irradiation with 400 or 500 rad

Group	% Uptake of ^{59}Fe	
	Spleen	Bone marrow
A. Unirradiated control	7.01 ± 0.6	0.59 ± 0.07
B. Control 400 rad	9.91 ± 0.8	1.37 ± 0.10
C. Leucotrofina (5u/day × 6) after 400 rad	13.31 ± 0.3[a]	1.84 ± 0.15[b]
D. Control 500 rad	5.35 ± 0.3	0.64 ± 0.06
E. Leucotrofina (5u/day × 6) after 500 rad	10.30 ±0.5[b]	1.01 ± 0.06[b]

Statistical significance
[a] $p < 0.05$ and [b] $p < 0.01$

The amount of DNA in the thymus (Fig. 1) and the uptake of ^{3}H-thymidine into thymic DNA (Fig. 2) were strongly enhanced following irradiation with 500 rad in Leucotrofina-treated A2G mice. The recovery effect

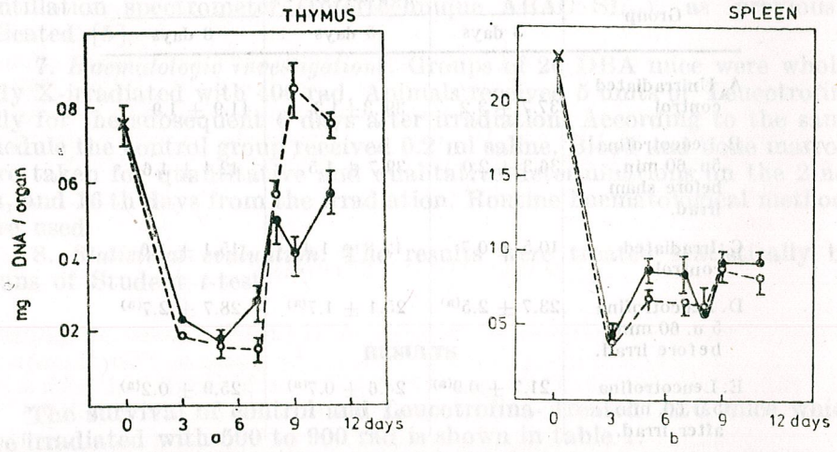

Fig. 1. — Amount of DNA in the thymus (a) and the spleen (b) of whole-body X-irradiated A2G mice (500 rad), with (○) or without (●) the application of Leucotrofina (see Materials and methods). Non-irradiated control group (×).

was also evident in the bone marrow after the application of Leucotrofina (Fig. 3). However, no differences were found in DNA content and ^{3}H-thymidine incorporation into spleen DNA of control and Leucotrofina-treated A2G mice (Figs. 1 and 2). The dynamics of haematologic changes in the

peripheral blood and in the bone marrow are presented in table 4. As shown in table 4 the dynamics of the recovery processes in both compartments (peripheral blood and bone marrow) are more marked in Leucotrofina-treated animals.

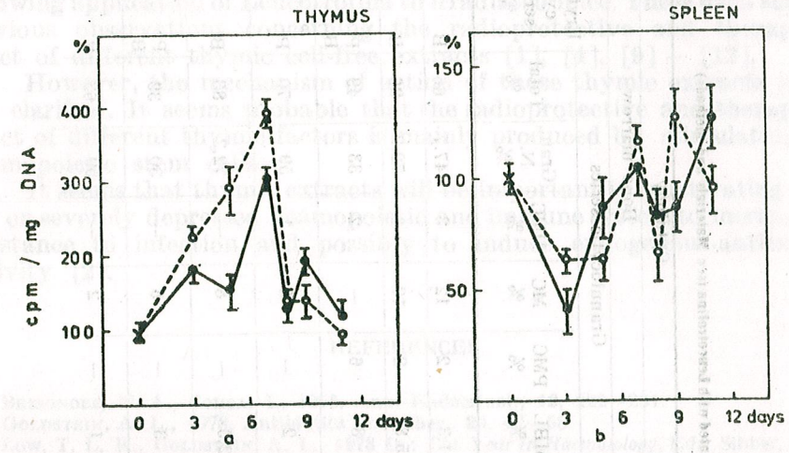

Fig. 2. — Specific activity of DNA in the thymus (a) and the spleen (b) of whole-body X-irradiated A2G mice (500 rad) after the administration of 12 μCi of ^{3}H-thymidine at 90 minutes before killing. Mice were irradiated with (○) or without (●) the application of Leucotrofina. Specific activity in unirradiated mice (×) was of 22,450 cpm/mg DNA in the thymus and 91,300 cpm/mg DNA in the spleen.

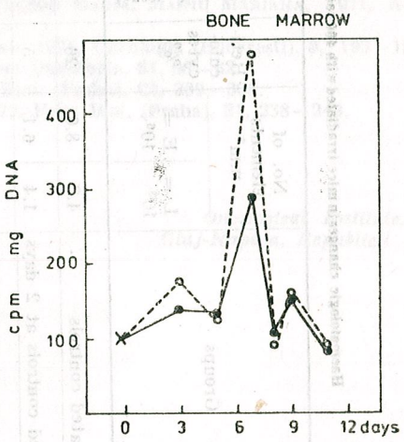

Fig. 3. — Specific activity of DNA in the bone marrow. A similar experiment as that described in Fig. 2. Symbols as in Fig. 2 Specific activity in the unirradiated group was 69,300 cpm/mg bone marrow. DNA

DISCUSSION

The data presented in this work show that the post-irradiation treatment of whole-body mice irradiated with Leucotrofina, a cell-free thymic extract, was associated with an increased survival time (table 1).

Table 4

Haematologic changes in mice irradiated with 400 rad, untreated and treated with Leucotrofina (see Materials and methods)

No.	Groups	No. of elements mm³		Cytology														Lymphocytic series %
				Peripheral blood			Bone marrow											
							Granulocytic series									Erythrocytic series %		
		L = 10^3	E = 10^6	Reticulocytes %	Gran. N. %	Lymph. %	MBL %	PMC %	MC %	MMC %	Gran. N. %	Total %						
1	Unirradiated controls	4.7	8.6	26	28	72	1.3	2	12	3	47.7	66				B	26	8
2	Irradiated controls at 2 days	1.4	6.1	5	57	43	5.8	2	10	6	72.2	96				B	3	1
3	Irradiated controls at 9 days	1.6	8.0	31	30	70	8	6	—	2	33	49				P B	5 39	7
4	Irradiated controls at 16 days	5.1	9.1	46	36	64	2	—	8	1	59	70				Pr	22	8
5	Leucotrofina (2 × 0.2 ml) after irradiation at 2 days	1.5	6.7	6	86	14	2.3	—	8	—	72.2	83				B	13	4
6	Leucotrofina (6 × 0.2 ml) after irradiation at 9 days	3.8	8.9	63	32	68	3	—	6	—	30	39				Pr B	7 48	6
7	Leucotrofina (6 × 0.2 ml) after irradiation at 16 days	5.6	9.1	26	35	65	1	—	2	—	59	63				P	27	10

B = Basophil; P = Polychromatophil; Pr = Proerythroblast.

A faster haemopoietic recovery was noted by ^{59}Fe uptake into erythrocytes (table 2), spleen and bone marrow (table 3) and by haematological investigations (table 4) of Leucotrofina-treated mice. Lymphopoiesis was also enhanced in the thymus (Figs. 1 and 2) and in the bone marrow (Fig. 3) following application of Leucotrofina to irradiated mice. These data support previous observations concerning the radioprotective and therapeutic effect of different thymic cell-free extracts [1], [4], [9] — [12].

However, the mechanism of action of these thymic extracts is still not clarified. It seems probable that the radioprotective and therapeutic effect of different thymic factors is mainly produced by stimulating the haemopoietic stem cells.

It seems that thymic extracts will be important in reactivating anergic or severely depressed heamopoietic and immune system to increase the resistance to infection and possibly to induce endogenous-antitumour activity [2].

REFERENCES

1. Bezsonoff, N. A., Comsa, I., 1958. Ann. Endocrinol., **19**, 222—231.
2. Goldstein, A. L., 1978, Antibiotics Chemther., **24**, 47—58.
3. Low, T. L. K., Goldstein, A. L., 1978 In: *The Year in Haematology*, Ed. Sibber, Plenum Publishing Corporation, 281—292.
4. Potop, I., Milcu, S. M., 1973, In: *Thymic Hormones*, Ed. Luckey, T. O., University Park Press, Baltimore, London, Tokyo, 205—271.
5. Suciu, D., Uray, Z., Abraham A. D., 1975, Int. I. Radiat. Biol. **28**, 409—416.
6. Suciu, D., Uray, Z., Mariana Maniu, 1976, Int. I. Radiat. Biol., **30**, 409—417.
7. Till, I. E.,, McCulloch, E. A., 1961, Radiat. Res., **14**, 213—218.
8. Uray, Z., Fărcășanu, M., Ursu, G., Onișor Maria, Maniu Mariana, 1971, Atomkernenergie, **18**, 327—328.
9. Uray, Z., Mariana Maniu, Camelia Ban, 1978, Oncologia (București), **3**, 193—198.
10. Uray, Z., Mariana Onișor, 1977, Magyar Onkologia, **21**, 60—62.
11. Vavrova, I., Petirek, P., 1976, Folia Biol. (Praha), **22**, 289—308.
12. Vavrova, I., Petirek, P., Mraz, I., 1975. Folia Biol. (Praha), **21**, 238—243.

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RESPONSE OF *IPS TYPOGRAPHUS* L. (COLEOPTERA, SCOLYTIDAE) TO AGGREGATION PHEROMONE IN MIXTURE WITH ALPHA PINENE

BY

N. TOMESCU, LUCIA DUSA, GH. STAN, I. OPREANU, F. HODOȘAN and LEONTINA TĂUȚAN

The authors have studied the attractiveness of aggregation pheromone compounds in *Ips typographus*. Fifteen different mixtures were bioassayed: combinations of the pheromone compounds and alpha pinene-monoterpene produced by the host plant.

The greatest attractiveness was displayed by the mixtures of *cis*-and *trans*-verbenol, ipsdienol, methylbutenol and alpha pinene. The most important compound of the aggregation pheromone seems to be methylbutenol, since its absence has determined an important decrease of the attractiveness of the other compounds. Alpha pinene is not attractive either to males or to females; it has a synergic role in mixture with the aggregation pheromone. It seems that ipsenol is a repressor only for females; its presence in the mixture did not modify the males' response. In most cases the females were attracted in a greater number than the males.

The researches of the last two decades carried on with numerous Scolytidae species have undoubtedly demonstrated the role of chemical substances (pheromones and plant-produced substances) in the beetles' attack upon some new hosts [1], [3], [6]. The selection of the host by the beetles starting the attack (primary attraction) and the attracting of a part of the population until the optimal density and sex ratio (secondary attraction) are achieved, are based — to a great extent — on chemical stimuli [3].

This paper presents the results of our researches under laboratory conditions on *Ips typographus*, concerning the attractiveness of some aggregation pheromone compounds (*cis*-verbenol, *trans*-verbenol, ipsenol, ipsdienol and methylbutenol), combined in different variants both between themselves and with alpha pinene (host plant-produced monoterpenes).

MATERIAL AND METHOD

The biological material used in the experiments was brought from a spruce fir forest of the Someș Valley. Spruce fir trunks attacked by *Ips typographus* were cut and carried to the laboratory where they were kept in special cages at 24°C until the beetles'complete development. The adults, attracted by the light, left the trunks and were collected in nylon bags. In most cases the beetles were bioassayed the day they were collected. In a few cases they were stored at + 10°C for 2 — 3 days, after which they were used in the experiments. The data of literature show that the bark beetles may be kept at low temperatures (+ 8 — + 10°C) for 10. — 15

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days without any change of their behaviour [4], [7]. For the experiments we selected the beetles having a good physical condition, taking into account the vigour of their shifting. Before the experiments, the beetles were kept in big glass cages at + 24°C for 3 — 4 hours, in natural light; during this time they could make short flights. The testings took place in a bioassay room, at + 24°C temperature, about 66% humidity and 35 — 40 lux/m² (red light) light intensity. For each substance about 40 beetles in groups of 5 specimens were bioassayed. Each group was subjected to a test only once. Since there are no external morphological characters allowing to recognize the males and the females, the identification of sexes can be done only after dissection under the stereomicroscope. Therefore, after the bioassay, all the beetles were dissected in order to establish the ratio of males and females used in the experiments. The bioassays were made by means of a Wood-Bushing type olfactometer [8], partly modified [4]. The bioassayed substances were released at a constant rate of 2 µl/hour, in an air flow of 100 cc/min. An automatic microsyringe was used [4]. We bioassayed a total of 15 mixtures composed of: *cis*-verbenol (56.6%), *trans*-verbenol (91%), ipsenol (90%), ipsdienol (95%), methylbutenol (8% in alcohol solution) and alpha pinene (100%). Excepting the methylbutenol, all the substances were mixed in the proportions shown in table 1. The methylbutenol — being under the form

Table 1

Substances bioassayed under laboratory conditions

No. of bioassayed substances	cis-v. (A)	trans-v. (B)	ipsdienol (C)	ipsenol (D)	methylbutenol (E)	alpha-pinene (F)
I	1	1	0.2	—	—	—
II	—	—	—	—	—	1
III	—	—	—	—	1	—
IV	—	—	—	—	1	1
V	1	1	1	1	—	1
VI	1	1	1	1	1	1
VII	1	1	1	—	1	1
VIII	1	—	1	—	1	1
IX	1	—	1	—	1	—
X	1	—	1	—	20	—
XI	1	—	1	—	20	1
XII	1	1	1	—	20	—
XIII	1	1	1	—	20	1
XIV	1	1	0.2	—	20	1
XV	1	—	0.2	—	20	1

of an alcoholic solution in a concentration of only 8% — was put on filter paper in amounts corresponding to the concentrations in the table. The filter paper was placed in a glass tube where the needle of the microsyringe was introduced.

RESULTS AND DISCUSSIONS

The researches of Vité and coll. [5] have demonstrated the existence of 4 compounds of aggregation pheromone in *Ips typographus*: *cis*-and *trans*-verbenol (major compounds), ipsenol and ipsdienol (minor compounds). Bakke and coll. [2] established the existence of a new compound, the methylbutenol. The investigations in the field [2] showed that the isomers of verbenol and methylbutenol have an important role in the aggregation in *Ips typographus*. In our laboratory investigations we observed that the males' and females' responses to the bioassayed mixtures were relatively similar. Most of the substances have a stronger attractiveness for females (table 2, Fig. 1), a fact which has a logical, causal expla-

Table 2

Response of *Ips typographus* to different mixtures of aggregation pheromone compounds and alpha pinene

Bioassayed substance	Total no. of beetles	Of which		Positive response		Percentage %	
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
I	40	23	17	1	—	4.5	—
II	48	19	29	—	—	—	—
III	40	25	15	6	1	24	6.6
IV	40	15	25	5	12	33.33	48.0
V	40	25	15	—	3	—	20.0
VI	32	20	12	12	6	60.0	50.0
VII	40	26	14	16	10	61.53	71.42
VIII	50	22	28	12	17	54.5	60.7
IX	50	32	18	16	10	50.0	55.55
X	48	25	23	11	12	43.47	52.17
XI	50	26	24	12	12	46.15	50.0
XII	50	19	31	8	23	42.10	74.19
XIII	46	30	16	18	14	60.0	87.5
XIV	47	23	24	14	18	60.86	75.0
XV	40	25	15	7	10	31.81	43.0

nation. It is the males who start the attack and secrete the aggregation pheromone by means of which they attract and concentrate a part of the population on the new host. Most of this part of the population is composed of females. The high receptivity of females to the aggregation pheromone is a useful adaptation feature of the beetle populations, which

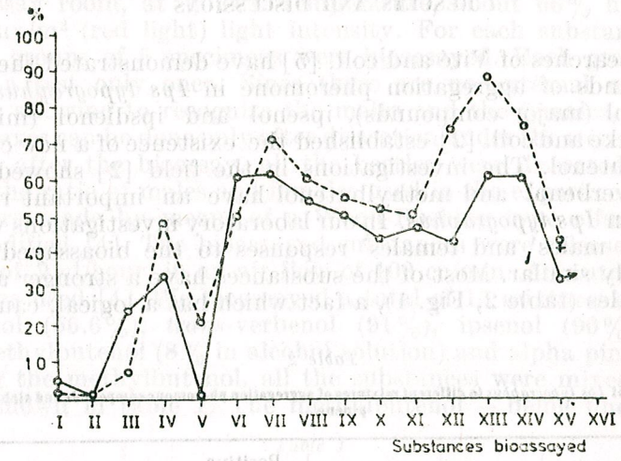

Fig. 1. — Attractivity of aggregation pheromone compounds in *Ips typographus* in combination with alpha-pinene.

assures the achievement of a proportion of 1 : 3 — 1 : 5 between males and females in the colonization period on a new host, a proportion which is characteristic of the genus.

The highest attractiveness was recorded by the mixtures composed of *cis*-and *trans*-verbenol, ipsdienol, methylbutenol and alpha pinene. The absence of methylbutenol from the mixture led to a marked decrease of the males' response (Fig. 1. subst. V). Similar results were recorded in the bioassays of mixtures without *trans*-verbenol (subst. VIII and XI), *trans*-verbenol and alpha pinene (subst. IX and X), alpha pinene (subst. XII), as well as in the case of the mixture where *trans*-verbenol was absent and the ipsdienol concentration was reduced to 1/5 (subst. XV). The simple decrease of ipsdienol concentration, without the modification of the other compounds, does not influence the males' response (subst. XIV). Deprived of attractiveness was also the mixture composed only of *cis*-, *trans*-verbenol and ipsdienol (subst. I).

The alpha pinene, bioassayed alone, proved to be deprived of attractiveness both for males and females (Fig. 1. Subst. II). On the contrary, the methylbutenol (subst. III) had a higher attractiveness in males. The bioassay of the two substances in mixtures with the other compounds of the aggregation pheromone demonstrates their synergic role, particularly with methylbutenol.

In females the attractiveness of the mixtures was similar with that present in males; the difference was that, generally, the percentage of

the females answering to the bioassayed substances was higher than that of the males, reaching its maximum value in the mixture where the compounds were present in a proportion of 1 : 1, excepting methylbutenol, which was in a proportion of 20 : 1 (subst. XIII). The presence of ipsenol, even mixed with methylbutenol (subst. VI), provoked a decrease of the females' response. It is possible for ipsenol to have a repressor role only upon the *Ips typographus* females, contributing in this way to the numerical regulation of the males and females on the newly attacked host.

Based on our results, we may conclude that in the secondary attraction in *Ips typographus* an important part is played, first of all, by methylbutenol, then by the isomers of verbenol and ipsdienol. The four compounds manifested a high attractiveness only when they were mixed in a proportion of 1 : 1. In females, a higher concentration of methylbutenol has determined an increase of attractiveness. The host plant-produced alpha pinene monoterpene has a synergic role in the secondary attraction and probably in the primary one, too, since when it was bioassayed alone it was not attractive for either males or females.

REFERENCES

1. Bakke, A., 1970, Contrib. Boyce Thompson Inst., **24**, *3*, 309—10.
2. Bakke, A., Froyen, P., Skattebol., L., 1977, Naturwissenschaften, **64**, 135.
3. Borden, J., 1967, Can. Ent., **99**, *11*, 1164—93.
4. Tomescu, N., Clark, E., White, J., Thomas, H., 1978, Trav. Mus. Hist. nat. „Grigore Antipa", **19**, 185—8.
5. Vité, P. J., Bakke, A., Renwick, A. A. J., 1972, Can. Ent., **104**, *12*, 1967—75.
6. Vité, P. J., Francke, W., 1976, Naturwissenschaften, **63**, 550—5.
7. Werner, A. R., 1972, J. Insect. Physiol., **18**, 1403—12.
8. Wood, L. D., Bushing, W. R., 1963, Can. Ent., **95**, *10*, 1066—78.

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CENTENAIRE DU PARASITOLOGISTE IOAN CIUREA ET DE L'HISTO-ENTOMOLOGISTE CONSTANTIN N. IONESCU

Sous les auspices de la Section des sciences biologiques de l'Académie de la République Socialiste de Roumanie furent organisées les manifestations de l'anniversaire du centenaire du parasitologiste I. Ciurea et de l'histo-entomologiste C. N. Ionescu dans la salle de conférences du Muséum d'Histoire Naturelle « Gr. Antipa », le 7 décembre 1978.

I. Ciurea (1878—1944) étant un des fondateurs de la parasitologie roumaine, sa vie et différents aspects de son œuvre scientifique furent évoqués par les prof. Al. Niculescu et I. Bercea de la Faculté de Médecine Vétérinaire, où le prof. I. Ciurea a autrefois déployé sa féconde activité. Prof. Elena Chiriac de la Faculté de Biologie a relevé ses mérites de promoteur en parasitologie écologique par ses recherches concernant surtout les cycles évolutifs des Trématodes Digènes transmis aux oiseaux du Danube et du Delta, aux mammifères et à l'homme (tel *Opistorchis felineus*) par suite d'un régime ichtyophage. I. Ciurea a joui de la haute estime du grand helmithologiste K. J. Skrjabin, dont le centenaire vient également d'être commémoré en l'Union Soviétique. Les docteurs Gh. Olteanu et Laurențiu Georgescu ont retracé d'autres préoccupations parasitologiques d'I. Ciurea, notamment ses études sur les attaques des simulies.

La vie et l'œuvre de C. N. Ionescu (1878—1935) ont fait l'objet des communications des professeurs V. Gh. Radu de l'Université de Cluj-Napoca, M. A. Ionescu et Tr. Orghidan des institutions biologiques de Bucarest. Disciple de l'école de morphologie animale du prof. P. Bujor à l'Université de Jassy et d'E. Haeckel à Jena, C. N. Ionescu est continuellement présent dans la bibliographie scientifique internationale par ses recherches approfondies sur la structure comparée de l'encéphale chez les différentes castes d'abeille (mâle, femelle féconde, ouvrière), que l'on est actuellement porté à mettre en corrélation avec leur étonnante capacité de communication signalétique. Ultérieurement, C. N. Ionescu fut le premier à explorer les grottes de Roumanie et à en décrire la faune de collemboles, ajoutant ainsi une contribution originale à la biospéologie qu'E. G. Racovitza venait d'édifier par ses travaux en France.

COMMÉMORATION DU BICENTENAIRE DE LA MORT DE CH. LINNÉ

Le retentissement puissant de l'ordre classificateur introduit par Ch. Linné (1707—1778). dans les sciences biologiques a fourni la base des exposés réunis sur l'initiative de la Section des sciences biologiques de l'Académie de la République Socialiste de Roumanie, afin de rendre hommage à la mémoire de l'illustre naturaliste suédois. Cette manifestation a également eu lieu dans la salle de conférences du Muséum d'Histoire Naturelle « Gr. Antipa », le 22 décembre 1978. Prirent successivement la parole le prof. Tr. I. Ștefureac qui donna un aperçu de la vie mouvementée et de la magnifique œuvre botanique de Ch. Linné ; le prof. M. A. Ionescu qui a dégagé la signification de l'œuvre linnéenne dans la classification du règne animal, y compris l'homme et sa place dans la nature. Finalement, V. Săhleanu a mis en relief l'apport considérable de Ch. Linné à la pensée scientifique moderne, sa contribution à l'éclosion de l'évolutionnisme et l'intérêt constant de la systématique en tant que moyen d'intégration des connaissances biologiques.

Acad. Radu Codreanu

Probleme de biologie evoluționistă — Taxonomie și speciație (Problèmes de biologie évolutionniste — Taxonomie et spéciation), sous la direction de R. CODREANU, Ed. Academiei, Bucarest, 1978, 178 p., illustr.

Le volume est un recueil de 31 articles consacrés aux problèmes de biologie évolutive, notamment à la spéciation et aux aspects fondamentaux de la taxonomie, qui ont été exposés et discutés dans un symposium organisé sous les auspices de l'Académie de la République Socialiste de Roumanie et de l'Institut des Sciences biologiques de Bucarest. Ces travaux sont groupés en cinq sections : zoologie et évolutionnisme, taxonomie et spéciation, chémotaxonomie, taxonomie animale, taxonomie végétale. La plupart des articles sont des mises au point des problèmes majeurs de l'évolutionnisme en rapport avec la taxonomie évolutive : relations entre ontogenèse et phylogenèse, concept biologique de l'espèce, principes de la taxonomie numérique, chémotaxonomie, taxonomie des virus, place de la taxonomie dans la biologie moderne, ses rapports et ses incertitudes, critériums éthologiques à l'appui de la taxonomie, particularités des espèces apomictiques. Ce sont des synthèses basées sur les données les plus récentes de la bibliographie évolutionniste et renfermant également les résultats des recherches et des méditations des auteurs.

D'autres articles traitent de problèmes plus spéciaux, tels la valeur des caractères chez certains groupes d'animaux (acariens, mysidacés récents et fossiles) et de plantes (champignons, bryophytes), l'importance des collections zoologiques et des herbiers, les règles de nomenclature. L'article sur le concept de l'espèce chez les hyménoptères parasites indique la part de l'hybridation interspécifique dans la spéciation de ces insectes. On expose en outre les tendances nouvelles en biospéologie, certains aspects de la protection de la nature et de la lutte biologique contre des espèces nuisibles. Un article développe des considérations biométriques sur l'évolution d'un copépode et un autre signale des anomalies chromosomiques dues aux pesticides.

Retenons l'orientation moderne des contributions qui nous offrent le choix entre la taxonomie numérique, la phylogénie dichotomique de W. Hennig et les concepts de la théorie synthétique de l'évolution. L'avant-propos de ce livre souligne la complémentarité qui doit nécessairement relier l'analyse expérimentale des faits actuels de spéciation aux processus historiques de phylogenèse que révèle la taxonomie. Le but du présent volume est justement d'alguillonner les lecteurs vers une compréhension approfondie des problèmes de l'évolution.

Doina Codreanu-Bălcescu

Probleme de ecologie terestră (Problèmes d'Ecologie terrestre) (sous la direction de R. Codreanu), Ed. Academiei, Bucarest, 1978, 259 p., illustr.

Ce volume renferme les travaux d'un symposium d'Ecologie terrestre, organisé sous l'égide de la Section des sciences biologiques de l'Académie de la République Socialiste de Roumanie et dédié à l'anniversaire du centenaire du professeur Andrei Popovici — Baznoșanu (1876—1969) qui fut un initiateur du mouvement écologique roumain et de la protection de la nature. Le volume débute par deux articles, destinés à évoquer la personnalité et l'œuvre zoologique et botanique de ce maître qui a puissamment influencé le progrès des sciences biologiques en Roumanie. Après un texte général sur la distinction à faire entre aire de répartition géographique et écologique, les 32 articles qui suivent sont groupés selon leurs affinités en cinq sections intitulées : Ecologie du sol, Ecologie des plantes, Ecologie animale, Ecologie des animaux nuisibles, Effets de pollution.

L'esquisse d'une classification écologique des sols en Roumanie précède des données pédologiques appliquées à l'agriculture et à la viticulture. La section d'écologie végétale réunit des articles sur des écosystèmes de prairie et sur les caractéristiques de certaines phytocénoses forestières et de leur productivité. On note une diversité plus ample des sujets d'écologie animale portant sur différents groupes, depuis les gastropodes et les isopodes terrestres, à travers divers insectes tels les collemboles, termites, chrysomélides, formicides, races écologiques de culicides, jusqu'aux oiseaux du Delta et aux spécimens captifs des jardins zoologiques. Concernant l'écologie des espèces nuisibles, sont envisagées les relations entre les insectes qui attaquent les forêts, la vigne, le verger, des arbustes ornementaux, le maïs, le pavot et

leurs entomophages ainsi que les méthodes biologiques à les combattre. En outre, trois articles sont consacrés à l'écologie des populations de rongeurs des agrosystèmes Le volume s'achève par l'analyse des déséquilibres écologiques dus à l'usage des pesticides et des insecticides.

Par l'alliance des aspects écologiques théoriques et appliqués des cas variés des productivité biologique, ce livre répond à des besoins actuels du développement de nos connaissances scientifiques.

Doina Codreanu-Bălcescu

XENIA G. SCOBIOLA-PALADE, *Tenthredinoidea-Tenthredinidae (Selandriinae, Tenthredininae, Heterarthrinae)*, in *Fauna Republicii Socialiste România*, București, Ed. Academiei, 1978, vol. 9, fasc. 8, 248 p., 176 fig.

L'auteur trouve en étudiant le matériel rassemblé par soi-même et les collections du Muséum d'Histoire Naturelles « Gr. Antipa » de Bucarest et du Musée de Sciences Naturelles de Sibiu, que sur le territoire de la Roumanie vivent 54 espèces (dont 6 à existence présumée) de *Selandriinae*, 124 espèces (dont 10 présumées) de *Tenthredininae* et 5 espèces de *Heterarthrinae*.

Les adultes de la plupart de ces hyménoptères étant nectarivores peuvent fortuitement actionner comme pollinisateurs des plantes visitées, ou bien quelques-uns, prédateurs sur de petits coléoptères et diptères, peuvent parfois consommer les nuisibles.

Leurs larves, phytophages, sont pourtant nuisibles, produisant parfois lors de la multiplication en masse de l'espèce de sérieux dégâts dans les divers champs cultivés, potagers, jardins, vergers, prés, bois de Roumanie.

La partie générale du fascicule s'occupe succinctement des caractères morphologiques nécessaires à l'identification des tenthrèdes, des aspects de la reproduction et du développement, des données paléontologiques et phylogénétiques, de la distribution géographique, des méthodes de collection et de recherche. Dans la partie systématique, après la caractérisation des *Tenthredinidae* suit la clé de détermination de ses 5 sous-familles, dont 3 seulement sont traitées dans ce premier fascicule. Après une brève mais claire caractérisation morphologique de chacune de ces sous-familles suit la clé de détermination de leurs tribus respectives. La caractérisation de chaque tribu est suivie d'une clé de détermination de ses genres et ceux-ci sont munis de la clé de détermination des espèces respectives. Basées sur de caractères nets, ces clés sont facilement accessibles.

Toutes les dénominations taxonomiques (familles, sous-familles, tribus, genre, espèces) sont suivies du nom de l'auteur et de l'année de leur première mention. Pour chaque espèce l'auteur décrit les adultes des deux sexes, parfois la ponte, les larves, l'écologie (mentionnant les plantes nourricières des larves et la période de vol des adultes), la distribution géographique générale et en Roumanie. Il y ajoute une riche illustration (dont de nombreux dessins originaux) concernant les détails de conformation de la tête, des ailes, de la dentelure des lames de scie, des sclérites de l'organe copulateur ou bien des valves péniales.

Vu l'abondance d'information exacte présentée, il résulte que l'auteur a consciencieusement consulté la littérature spécialisée, rendant ainsi un réel service aux agronomes et aux silviculteurs, comme à tous ceux préoccupés de problèmes d'écologie ou de biologie.

Victoria Iuga-Raica

DIMITRIE RADU, *Păsările lumii (The birds of the world)*, Edit. Albatros, București, 1977, 247 pages + 40 coloured plates, 786 figs.

This comprehensive work, dealing with some 10 per cent of the 8 600 species which make up the avifauna of the earth, discusses the representative types of all planetary orders and families in point of aspect, adaptation and behaviour. The material is arranged in a systematic succession — from the less evolved birds (Ratitae) to the best developed ones (Passeriformes), with special emphasis on the 356 species which populate permanently or temporarily Romania's avifauna ; original data are reported on their time of migration, nestling, distribution, density, a.o. As for the popular names foreign orders, families and species

go by, Radu Dimitrie makes either a translation from the denomination current in other languages or forges new terms based on the morphological or biological characters of the taxa described. The book provides ample information on the world of birds: after a short description of the morphological, anatomic, biological and ecological features of the bird class, the reader is shown the means of identifying birds and is given a systematic description of 3 subclasses, 32 orders and 148 families. Indexes of systematic classification of the depicted birds, of common and of scientific denominations are also included.

Despite its general informative character, the book offers many original data gathered by the author along years of painstaking ornithological research. His selection of the representative bird specimens, his clear style and synthetic presentation of the material and the importance he places on this homeothermal terrestrial vertebrates in maintaining the biological equilibrium of the planet enhance the value of this lavishly illustrated work, which fills up a gap in the Romanian speciality literature.

George A. Vasiliu

P. GROZA, R. CÂRMACIU, S. CANANĂU, V. FILCESCU, A. BORDEIANU, E DANELIUC, *Mediul ambiant natural și reactivitatea vegetativă* (L'environnement naturel et la réactivité végétative). II[e] vol., 230 p., 96 fig., Ed. Academiei, București, 1978

Une étude physiologique approfondie des réactions végétatives de l'homme exposé à des facteurs anormaux de l'environnement est attendue depuis longtemps. Ces facteurs exigent une forte sollicitation adaptative. En général, on a étudié le stress déterminé par les polluants qui posent des problèmes très actuels. Peu de travaux concernent l'action d'un milieu très chaud, tel celui des usines métallurgiques, ou bien l'action des états hypo- ou hyperbariques, comme à la montagne, en avion ou dans les mines ; ajoutons les modifications gravitationnelles, comme dans la submersion ou dans les vols cosmiques, etc.

Les auteurs se proposent d'étudier notamment les modifications adaptatives de la sphère végétative nerveuse ou endocrine chez des hommes qui travaillent dans les conditions plus haut mentionnées ou chez les animaux, en essayant de différencier les réactions spécifiques de celles non spécifiques (qui caractérisent le « stress » de Selye). L'action de la vasopressine (ADH) et de l'oxytocine sur les pressocepteurs, la liaision des centres hypothalamiques avec le système lymbique et les réactions affectives, la réponse cardiaque, celle des muscles lisses, l'équilibre hydrominéral, la fibrinolyse du sang, etc. ont constitué les tests fonctionnels.

Le premier chapitre présente l'activité du système réactionnel végétatif par un schéma très suggestif des facteurs naturels de l'environnement et de leurs récepteurs dans l'organisme.

Le deuxième chapitre traite de l'hyperthermie exogène, en donnant les résultats obtenus concernant les réactions cardio-vasculaires, la transpiration, l'hyperpnée, la salivation, l'action des médiateurs chimiques sur le centre thermorégulateur, la réaction sympatho-adrénergique, l'équilibre hydroélectrique et le rôle de l'oxytocine, de l'aldostérone, des hormones glycocorticoïdes, puis de l'uropepsine et de la fibrinolyse du plasma, à la suite des actions hyperthermiques de durées variables.

Le troisième chapitre s'occupe de l'hypoxie barique rencontrée aux altitudes montagneuses (espace possible pour le futur de l'humanité) et dans les vols aérospatiaux. Les modifications respiratoires et circulatoires, la syncope hypobarique, les modifications sanguines, le système endocrinien, l'équilibre hydrominéral, les échanges métaboliques, etc. sont étudiés.

Le dernier chapitre se réfère à l'action de l'hyper- et hypogravitation (immersion) sur la pression sanguine et sur les circulations locales dans des positions clino- et orthostatiques, sur les secrétions hormonales, sur l'équilibre hydro-minéral, sur la fibrinolyse, etc.

Le livre, paru sous la direction du professeur Petru Groza de l'Institut de Physiologie normale et pathologique de Bucarest, constitue un apport essentiel au problème du fonctionnement de l'organisme humain dans des conditions spéciales déterminées par certains facteurs du milieu, facteurs qui influent non seulement sur la vie végétative de l'homme, mais amènent des changements dans l'état psychique et émotionnel, avec la possibilité d'amélioration de ces états.

Une riche bibliographie suit chaque chapitre et un résumé en anglais termine ce livre qui est très utile aux médecins, aux biologistes et à tous ceux qui s'intéressent aux grands problèmes de la vie humaine contemporaine. Dommage que le livre ne soit pas aussi publié dans une langue de large circulation.

Eugen A. Pora

REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

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AVIS AUX AUTEURS

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

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

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

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, les tableaux et les graphiques sera évitée. Les références bibliographiques, citées par ordre alphabétique des auteurs, comporteront le nom de l'auteur, l'initiale du prénom, l'année, le titre de la revue, abrégé, conformément aux usances internationales, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé de 10 lignes au maximum. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). Le responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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