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REVUE ROUMAINE DE BIOLOGIE

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SOMMAIRE

CONTRIBUTION TO THE KNOWLEDGE OF THE FAMILY *PAGURAPSEUDIDAE* (*CRUSTACEA-TANAIDACEA*) OCCURRING IN THE INFRALITTORAL AREA OF THE WEST INDIAN OCEAN (TANZANIAN WATERS)

BY

MIHAI BĂCESCU

The author describes a new genus (*Macrolabrum*) and three species of Pagurapseudidae occurring in the infralittoral area of Tanzania, all belonging to the coral-reef biotope.

Three species of Tanaids * — e.g. Pagurians among Decapods — adapted to life in empty snail shells have been known so far. These are : *Pagurapseudes spinipes* Whitlegge (South Australia), *P. bouryi* Bouvier and *P. laevis* Menzies, the two latter ones occurring in the Caribbean Sea, all of them belonging to Fam. *Pagurapseudidae* Lang, 1971, emend, Băcescu, 1975.

Just like for other genera of Tanaidacea (Băcescu, 1975), the infralittoral area, populated with the rich coral-reefs of Tanzania, proved to be a particularly active evolutive center for *Pagurapseudes*, as well. This fact appears quite natural, given the constant and propitious biotope conditions and the presence of a high number of small-size Gastropods (Cerithidae, Phasianellidae, Neritidae etc.) developed on a sand layer derived, in the first place, from the grinding of dead corals. The different adaptative lines of Pagurapseudidae occurring in the Tanzanian infralittoral waters (to spongicolous, corallicolous, psammicolous life) led not only to an active speciation, but also to the creation of new genera, such as *Macrolabrum*, described by us hereunder and which is the most specialized type of the representatives of these shell-living Tanaids.

Macrolabrum n.g.

Diagnosis (♀). Tanaids adapted to the life in small shells of Gastropods (e.g. the species *Pagurapseudes*). Huge *epistome* extending much beyond the rostrum by its elongate, rough cone, ended with an apical spine.

A_1 with reduced flagella inserted at the same level ; inner flagellum unisegmented, outer one bisegmented, but with terminal segment short,

* Data concerning a fourth species, *Pagurapseudes guitarti* Guţu occurring in the Cuban waters are to be published.

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scarcely detectable, and a single aesthetasc. A_2 with strong segments, nearly equal in width. Uropods with unisegmented exopodite and uni- or bisegmented endopodite, both ended with a claw-seta, strong, doubly serrate in its terminal half; the setae are bent downwards forming typically climbing crochets resembling those found in some larvae of Trichoptera.

Rostrum large, linguiform, with a set of minute spinules. Chelae slightly unequal (♀).

1. **Macrolabrum trichopteroides** n. sp. (Fig. 1, *A*—*K*)

Description (♂, ♀). Small size Tanaid (1.5—1.8 mm), body with weak pilosity, only 1—2 hairs on the thoracomers and 2 hairs on each last pleonite. Abdomen 6-segmented, somewhat wider in females.

Rostrum large, linguiform, with frontal margin hatched by 8—12 small, conical tubercles (Fig. 1, *B*). Eyes well developed, with about 9 ommatidia, partially covered by a carapace fold, thus more clearly seen from the lateral view (Fig. 1, *A*).

The epistome—anterior projection of labrum—appears as a huge cone with pointed tip (*e* fig. 1 A), very striking, no matter the angle from which the animal is looked at; the labrum accounts for the genus name (Fig. 1, *A*); it is covered with tubercle-shaped scales. Among peraeopods — hyposphenia like some conical tubercles, provided with hairs. In ♂, a huge penial cone, as large as the basis of the relative peraeopod.

A_1 strongly developed, but poorly ornated (Fig. 1, *C*); only 2 spinulae on the proximal segment and one spinule on the median segment of the basis; flagella reduced; the inner flagellum to one segment, the outer one — a little longer and much broader — to two segments, the distal one being scarcely visible, one single aesthetasc (♂, ♀), simple, without terminal flagella or filaments (Fig. 1, *D*).

A_2 massive (Fig. 1, *E*) 5-segmented, with spines on the first 3 proximal segments — nearly equal — and with a minute terminal segment.

The meros and the long projection of the carpus of maxilliped are ended with a short seta.

Peraeopods I: chelae without exopodite, slightly assymmetrical in ♀ (Fig. 1, *A*); the right paeraeopod is a little larger; in ♂, heterochely more marked. Peraeopod II common to the family, with a dactylic claw relatively small and a very broad basis showing denticles and setae, over which a huge exopodite bents inwardly (Fig. 1, *F*, *f*) ending in a disk provided with 15—18 double pennate setae. The remaining peraeopods are common to the shell-living Tanaids. Prp. VII (Fig. *G*) has a reduced dactylic claw and 2 laminated phanerae (x, Fig. 1, *H*).

Pleopods with last segment like a discoidal blade with pennate setae all around.

Uropods with latero-terminal insertion are altogether different from the common structure in Apseudidae: the basis strong, short (cuboid), with unisegmented exopodite and a common unisegmented endopodite (Fig. 1, *J*), yet the symmetric uropod has a bisegmented exopodite; both rami, strongly bent ventrally, terminate in huge setae, curved and doubly serrate in the distal half, true sticking-climbing claws. The uropods do not show any difference between ♂ (Fig. 1, *K*) and ♀ (Fig. 1, *I* and *J*).

Size: 1.6 mm.

Color: brown-violet, especially in the posterior half of the carapace; even the abdomen is brown in contrast with the white marsupium or chelae. Eyes black, like chelae fingertips.

In the cylindrical marsupium, 4—6 embryos are strung after one another.

Material: 3 specimens (1♂, 2♀) collected from Makatumbe, Tanzania and 1 o of spongia *Cinachyra australiensis*, 30.XII.1973, collected from Mbudya, alongside with *P. varians*.

Holotype ♂, no 316 in the Crustacean collection of the "Gr. Antipa" Museum. Syntype, ditto, no 361.

Ecology. Remarks. Macrolabrum trichopteroides lives in the shells of Cerithidae in association with *P. varians* and differs from any Monokonophora, in the first place, by its huge epistome and by the *sui-generis* structure of the uropods. The latter are strongly bent downwards, resembling the claw of *Macrobiotus* among *Tardigrada* or of some Sericostomatidae, among Trichoptera, whence the species name. The genus is also characterized by its very short appendages. Flagella of A_1 stumpy.

Considering that the following new species of *Pagurapseudes* show many morphological features in common with the previous species, we give, for the moment, only brief diagnoses, as well as a dichotomic key for all the Tanzanian species; the figures are supplementing the description, facilitating their recognition. Thus:

2. **Pagurapseudes varians** n. sp. (Fig. 1, *L*—*P* and Fig. 2, *A*-*G*)

Diagnosis. Integument weakly calcified. Carapace with 6—7 lateral setae; rostrum — a large projection with parallel edges (Fig. 2, *A*); front straight or slighly prominent, provided with hyaline, small tubercles (Fig. 2, *B*), very various in number and arrangement, whence the species name (Fig. 2, *A*). A_1 (Fig. 1, *N*) with 3 spines on the basis; A_2 6-segmented (Fig. 2, *N*). The remaining appendages do not show essential differences (Fig. 1, *L*, *M*, *O*, *P*, 2, *C*—*G*).

Chelae shiny-white. Cephalothorax purplish blue-pink, golden eyes, where the brown ommatidia constellations are seen.

Material. Observations. Pagurapseudes varians is the most frequent and eurytope Pagurapseudid of the infralittoral area of Tanzania. Present in 18 out of the 28 stations with such Tanaids (in 3—4 stations, alongside with *P. dactylifrons*), in a number of 23—34 specimens.

— Makatumbe, 29.XII.1973, dredged at 25 m, sand and broken shells; 8 specimens in *Tricolia* and *Hydrobia* shells with yellow belt.

— Alongside the hundreds of snails dredged at 8 m in corals, at Mbudya; some 22 specimens were found with *P. dactylifrons* (in *Corralliophila*, *Tricolia* and in a small Naticid).

— Between Mbudya and Kunduchi, 15 m, muddy sand, 23.XII.1973, over 30 specimens (mainly from Cerithidae) 4 ♂♂, 5 ♀♀ ovigerous, the remaining being females and ♂♂ juv. (+ 4 *P. dactylifrons*).

— Off the spongia *Cinachyra australiensis*, Mbudya, 1 m, 3.I.74, 2 ♀♀ ovigerous, 5 juv.,

— From a twisted, violet, cylindrical spongia, Logg Kabine, 20.XII.1973, 22 specimens — 13 ♀♀ 9 ♂♂ (9 ovigerous : with 7—9 embryos + 12 specimens of *P. dactylifrons*).

— Dredged in a *Cymodocea* field, 30.XII.1973, 5—8 m. Logg Kabine, 9 specimens (together with *P. dactylifrons*).

— Dredged in sand areas between the *Syringodium* fields, 0.50 m, upon ebbing, 11 specimens in *Tricolia* shells and in Cerithidae.

— From *Corallina* algae, dredged at 20 m E of Kunduchi, 29.XII. 1973, 4 specimens.

— Fallen off the *Pinna* epibiosis, live, in front of the Africana Hotel (S. Kunduchi), 16.XII.73, 3 specimens in *Cerithium*.

Holotype ♀, no 314, collection of the "Gr. Antipa" Museum; 10 syntypes ditto, no. 315.

3. **Pagurapseudes tricoliae** n. sp. (Fig. 2, *H*—*K*)

Diagnosis. Integument calcified, carapace rough in the anterior portion, with numerous excavated tubercles in the posterior third (Fig. 2, *H*); edges with spines in the antero-inferior part and 12—13 lateral setae. A_1 weakly armed; A_2 7-segmented (Fig. 2, *H*). Rostrum crown-shaped from the lateral view, with a triangular projection with 4 spines (Fig. 2 *H*). Maxilliped like in figure 1 *L*; Labium, a bisetose lobe (Fig. 2, *K*).

Peraeopod II : minute, does not extend beyond chela, with bisegmented exopodite ; terminal segment like a kidney-shaped blade (Fig. 2, *J*). Uropod like in figure 2, *I*.

The species is rare, large-sized (3—3.5 mm), living in *Tricolia* shells (Fam. Phasianellidae), whence the name.

Locality : Mbudya, sand, 8 m, 13.XII.1973.

Holotype ♀, no 317, in the Crustacean collection of the "Gr. Antipa" Museum.

Fig. 1, *A*—*K*. — *Macrolabrum trichopteroides* n.sp. ♀.*A*, anterior portion, lateral view; *B*, anterior portion, seen from above; *C*, A_1; *D*, its apical part, magnified; *E*, A_2; *F*, peraeopod II; *f*, its exopodite, magnified; *G*; peraeopod VII, with relative horacic segment and the penial tubercle on its sternal portion; *H*, peraeopod VII of another ♀; its distal portion, magnified. *I*, uropod ♀; *J*, ditto, seen from below; *K*, terminal part of abdomen, with left uropod, in usual position (♀). *L*—*P*, *Pagurapseudes varians* n.sp. *L*, maxilliped; *l*, its endite; *M*, flagella of A_1; *N*, A_2; *O*, epistome; *P*, abdomen and uropods. Scale of figure *B* also valid for *D*, *E*, *f*, *G*, *H*, *J* and *K*.

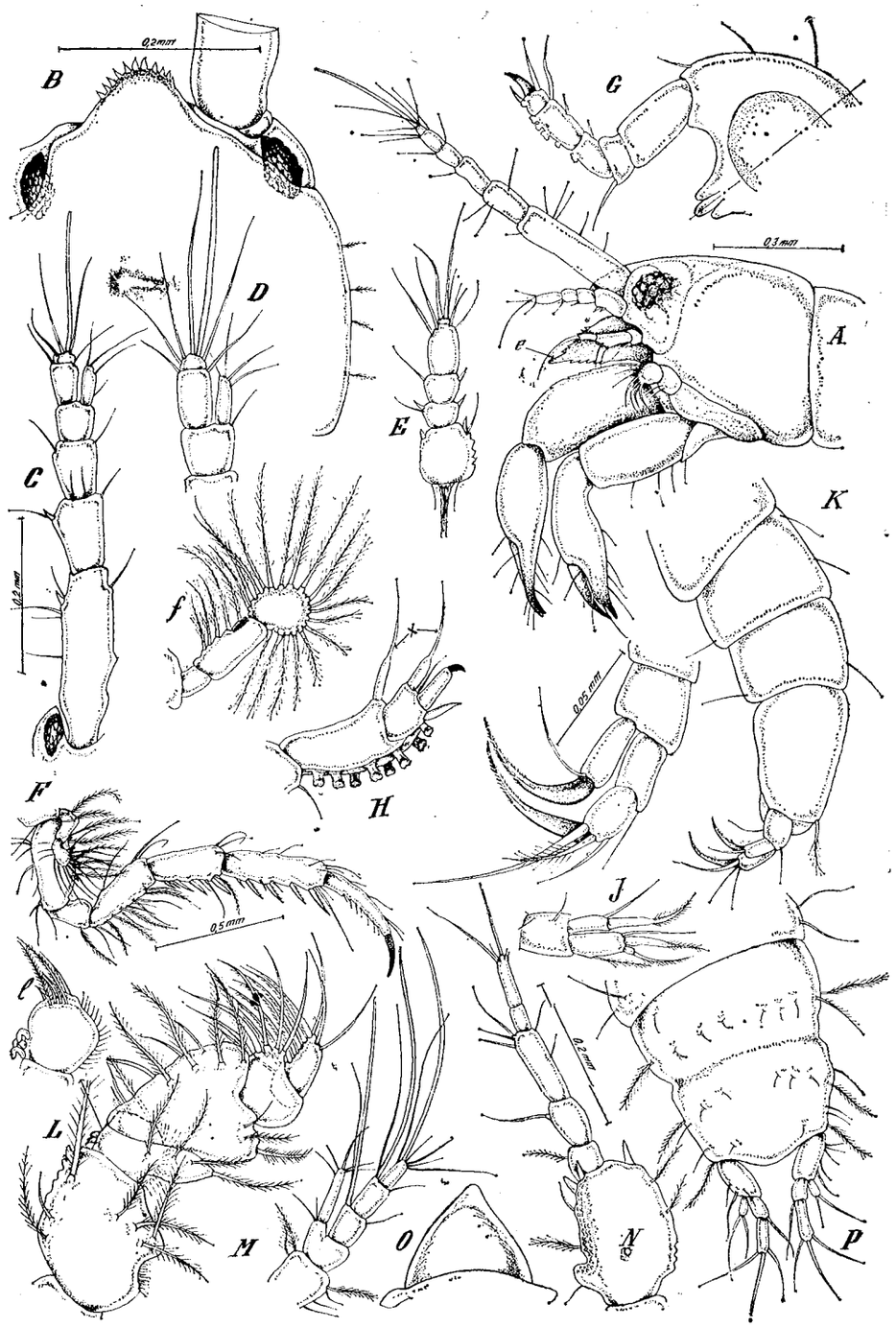

Fig. 1

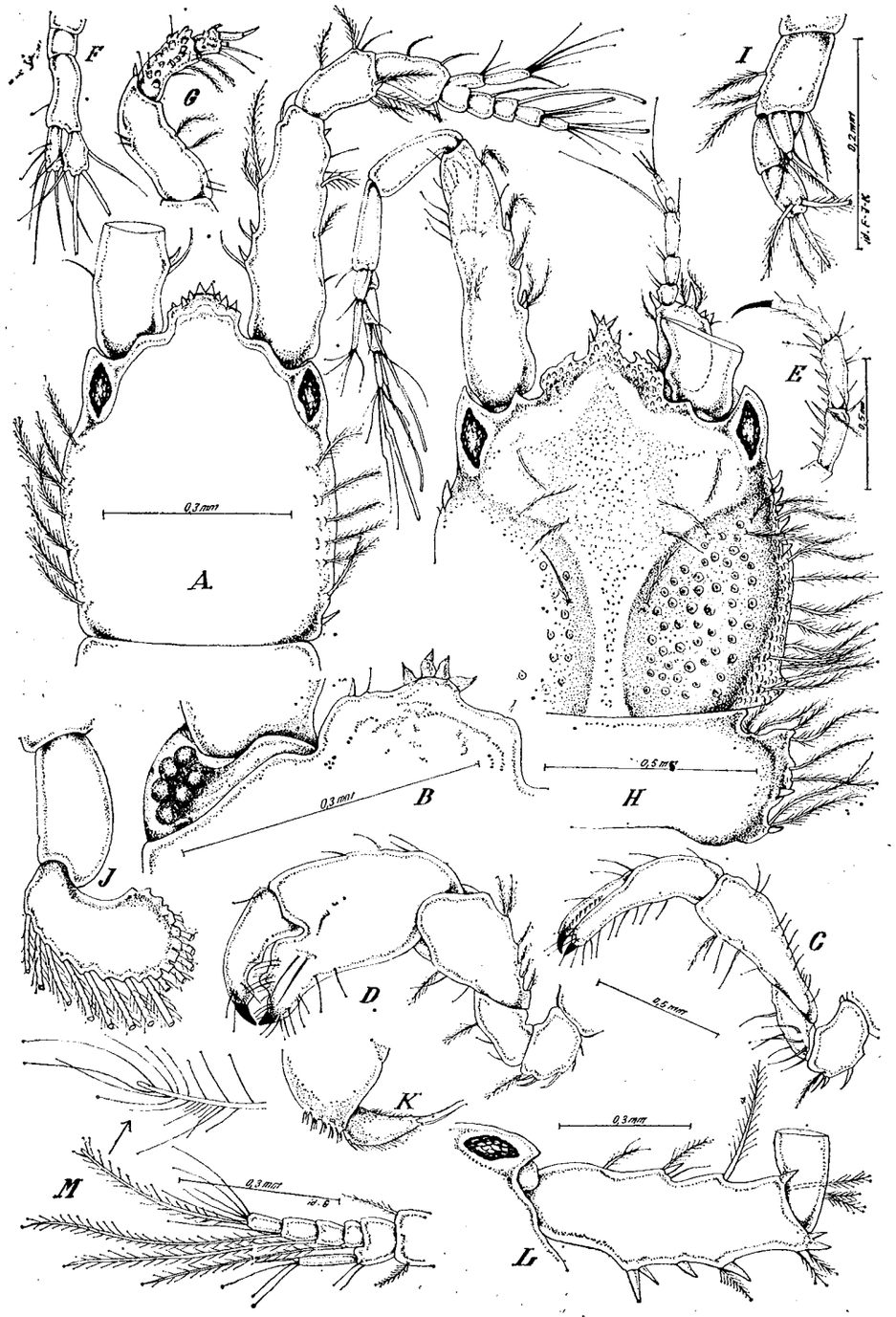

Fig. 2

Fig. 2. *A—G.* — *Pagurapseudes varians* n. sp. ♂ ♀. *A*, carapace, seen from above (♀); rostral portion of another specimen (♂); *C*, left chela of a ♂; *D*, ditto right chela; *E*, terminal portion of prp. II; *F*, pleopod ♂; *G*, prp. VII.
H—K, *Pagurapseudes tricoliae* n.sp. ♀. *H*, anterior portion of body (seen from above); *I*, uropod; *J*, exopodite of prp. II; *K*, labium with its bifid lobe.
L—M, *Pagurapseudes dactylifrons* Băc. *L*, basis of A_1: *M*, flagella of A_1 with filamentons aesthetascae; *arrow*, tip of an aesthetasc ♂ with long filaments. Scale of figure *N*, also valid for *C*, *L* and *l*, and that of figure *A*, valid for *M* and *P*.

DETERMINATION TABLE OF THE REPRESENTATIVES OF FAM. *PAGURAPSEUDIDAE* OCCURRING IN THE INFRALITTORAL AREA OF TANZANIA

1 (2). Outer flagellum of A_1 consisting of only 2 segments; the distal one minute, with a single, simple aesthetasc (Fig. 1, *C*); A_2 with segments ± equal in size (Fig. 1, *E*). Uropods terminating with 2 setae, strong, double serrate, claw-like hooked (Fig. 1, *J*). Epistome huge, thrice as long as the basis diameter (*e*, in : Fig. 1, *A*) . . . . . *Macrolabrum trichopteroides* n.g. n. sp.

2 (1). Outer flagellum of A_1 consisting of 2—4 segments, with 2—4 aesthetascae (Fig. 1, *M*); A_2 with a huge basal segment and 5—6 fine segments (Fig. 1, *N*). Uropods terminated with common, long, pennate setae, not curved (Fig. 1, *P*). Epistome small, as high as broad (Fig. 1, *O*) . . genus *Pagurapseudes* Menzies . .**3**

3 (4) Aesthetascae provided with long filaments (Fig. 2, *M*). Basis of A_1 with spines on both edges, some of the inner ones being double (Fig. 2, *L*). Rostrum like a tuft of spiniform projections, edged by 2 semicircles of small tubercles . . . . . *P. dactylifrons* Băc. (Bull. Sci. Fac. Dar es Salaam, 1976, *2*)

4 (3). Aesthetascae smooth, without filaments (Fig. 1, *M*). Basis of A_1 bearing 3 spinules only on the inner portion (Fig. 2, *A* and *H*). . . **5**

5 (6). Rostrum large with parallel edges and front straight or gently convex, bearing small, hyaline tubercles, either continuous or separated by gaps. Carapace smooth with 6—7 lateral setae, without latero-anterior spines (Fig. 2, *A*) . . . *P. varians* n. sp.

6 (5). Rostrum triangular with spines on the median projection and on the edges. Carapace very rough, with 10—12 lateral setae and with antero-inferior spines (Fig. 2, *H*) . . . *P. tricoliae* n. sp.

General remarks. None of the representatives of Pagurapseudidae from the Indian Ocean resembles the infralittoral group of species from the Caribbean Sea, the single area where these species have been thoroughly studied so far.

They mainly differ by the lack of an exopodite at the chelae and by the constant presence of a single pair of pleopods (♂, ♀) and of a huge biramous exopodite; a minute, uniramous one at prp. II (of the *Trichapseudes tridens* Barnard 1920 type) in the Tanzanian species, and in *P. spinipes* from the NS of Wales.

These two groups are certainly related to each other, yet they developed in 2 different evolutive lines.

REFERENCES

1. BĂCESCU M., 1975, *Archaic species of Tanaidacea from the Tanzanian waters, with the description of a new genus, Tanzanapseudes.* Rev. Roum. Biol. — Biol. Anim., **20**, 2, 81—91.
2. BĂCESCU M., *Three new genera and six new species of Monokonophora from the coral reefs of Tanzania (west Indian Ocean)*, Bull. Sci. Fac. Dar es Salaam, 1976.
3. BOUVIER E. L., 1918, *Sur une petite collection de Crustacés de Cuba offerte au Muséum par M. de Boury.* Bull. Mus. Nat. Hist. Nat., **24**, 6—15.

4. GUȚU M., 1972, *Phylogenetic and systematic considerations upon the Monokonophora* . . . Rev. roum. Biol. Zool. **17**, 5, 297—305.
5. LANG K., 1970, *Aufteilung der Apseudiden in vier Familien* . . . Ark. Zool. Stockholm, (2), **22**, 16, 515—625.
6. MAKKAVEEVA E. B., 1971, *Kacestvennti sostov Tanaidov rakov v Krasnom More.* Vol. Bentos Șelfa Krasnovo Moria, *Kiev*, 1971, 88—108.
7. MENZIES R. J., 1953, *The Apseudid Chelifera of the Eastern tropical and North temperate Pacific Ocean.* Bull. Mus. Compar. Zool. Harw. Univ. **107**, 9, 441—496.
8. WHITELEGGE T., 1901, *Crustacea Isopoda part 1, Sci. Res. Trawling Exped. H.M.C.S. "Thetis".* Mem. Austrat. Mus., *4*, 203—246.

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NOUVELLES ESPÈCES D'ORIBATES (ACARI : ORIBATEI)

PAR

MAGDA CĂLUGĂR et N. VASILIU

In the present work the authors describe two species new for science : 1. *Epilohmannia schusteri* n. sp. is characterized by a fusiform sensillus and very long and barbulated notogastral hair. 2. *Hypocepheus krivolutskyi* n. sp. is characterized by the absence of translamellae, by the aspect of lamellae, by the aspect alongated spatulated shape of the sensillus and by the notogastral ornementation with fossae.

La présente étude enrichit la connaissance du groupe des Oribates (*Acari* : *Oribatei* Dugés, 1833) par la description des deux espèces nouvelles collectées dans la région du bassin de Bahlui.

SUPERFAMILLE EPILOHMANNOIDEA Grandjean, 1969.

Famille **EPILOHMANNIIDAE** Oudemans, 1923.

1. Epilohmannia schusteri * n. sp.

Diagnose. Le sensillus fusiforme allongé, légèrement barbulé. Les poils notogastraux, fins, très longs et barbulés, à l'exception des poils : c_3, h_3, ps_3, qui sont aussi barbulés, mais très courts.

Dimensions. Prodorsum : L—253 μ ; l — 190 μ. Notogaster : L—475 μ ; l—348 μ. Volet génital : L—86 μ ; l—62 μ. Volet anal : L—130 μ ; l—31 μ. Poils du prodorsum : ss—75 μ ; la — 43 μ ; il — 111μ ; ro—31 μ ; ex—20 μ. Poils du notogaster : c_1—130 μ ; c_3—49 μ ; h_3, ps_3—22μ.

Morphologie. Le prodorsum présente les poils rostraux (*ro*) barbulés, courts, avec un socle bien visible, asymétriques. Les poils lamellaires (*la*), peu courbés, barbulés et orientés en avant, sont convergents. Les poils interlamellaires (*il*), qui dépassent en longueur toutes les paires de poils du prodorsum, sont peu courbés, barbulés et orientés en arrière. Tout près de la bothridie on trouve une paire de poils exobothriaux (*ex*) très petits et barbulés. Le sensillus (*ss*), fusiforme allongé, a sur sa surface de petites épines chitineuses. Le notogaster, oval-allongé, présente 14 paires de poils, dont 11 paires ont les poils très longs, peu courbés, fins et légèrement barbulés. Les trois autres paires de poils sont de dimensions réduites, se remarquant les poils ps_3 et h_3, qui représentent à peine

* Espèce dédiée au Dr R. Schuster, qui est également connu par une ample étude sur le genre *Epilohmannia* Berl., 1917.

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une moitié de la longueur et de l'épaisseur des poils c_3. Le corps présente sur la partie dorsale une ornementation ponctuée (fig. 1).

Sur la face latérale, on observe que le rostre ne couvre pas les chélicères et la partie antérieure du gnathosoma. La glande latérale est visible (fig. 2).

Sur la face ventrale, l'apodémata 1 est oblique et dirigée en arrière ; les apodémata 2 et 3 sont disposées presque parallèlement avec l'apodémata séjugale ; l'apodémata 4, oblique, est dirigée en avant. Les poils épiméraux barbulés et courts sont disposés d'après la formule : 3—1—2—3. Les volets génitaux, semi-circulaires, ont sept paires de poils fins, courts et barbulés. Les trois paires de poils agénitaux ont des poils courts, fins et barbulés. Les volets anaux, très bombés, et avec une position postérieure, portent trois paires de poils barbulés, qui sont plus longs que les génitaux. Il y a un anneau périanal plus élargi dans ses parties latérales. Des trois paires de poils adanales, qui sont les plus longs poils de la face ventrale, la deuxième paire est fixée sur l'anneau périanal. Ces poils sont aussi barbulés (fig. 3).

Le tarse IV a les poils *pv*, *s* et (*a*) comme des épines très courtes et épaissies et un seul poil *ft* long et barbulé (fig. 4).

Holotype, adulte et un paratype, chez les auteurs. Terra typica : le bois de chêne Păun, situé au S—E de la ville de Jassy. Biotope : la mousse. Date : le 9 septembre 1974 [1] [3] [5] [6].

Superfamille *CEPHEOIDEA* Balogh, 1961.

Famille **CEPHEIDAE** Berlese, 1866.

2. **Hypocepheus krivolutskyi** ** n. sp.

Diagnose. Les lamelles, qui ont les parties médianes élargies et pourvues d'une paire d'excrescences antéro-latérales, présentent des cuspis évidentes. La translamelle est absente. Les sensillus longs et spatulés. Six poils génitaux.

Dimensions. Idiosoma : L—545 μ ; l—372 μ. Poils du prodorsum : ro — 55μ ; la — 99 μ ; il — 150 μ ; ss—105 μ. Poils notogastrals : ta—86 μ ; ti—99 μ : ps_2, ps_3—45 μ. Volet génital : L—68 μ ; l—31 μ. Volet anal : L—99 μ ; l—49 μ.

Morphologie. Le prodorsum a les lamelles proéminentes qui s'élargissent dans leurs parties médianes où elles présentent une paire d'excrescences antéro-latérales. En avant, la lamelle se termine par un cuspis robuste qui porte un poil lamellaire (*la*) fin, long et avec le but légèrement convergent. La translamelle manque. Les poils rostraux (*ro*) sont les plus fins et courts parmi les poils du prodorsum. Au contraire, les poils interlamellaires (*il*) puissants, courbés et convergents, sont les plus longs poils du prodorsum. Les sensillus (*ss*), avec de longs pédicelles, présentent les parties terminales spatulées et pourvues de fins barbulés. Les bothridies globulaires sont réunies par une nervure transversale qui présente, anté-

** Espèce dédiée au Dr D. A. Krivolutsky.

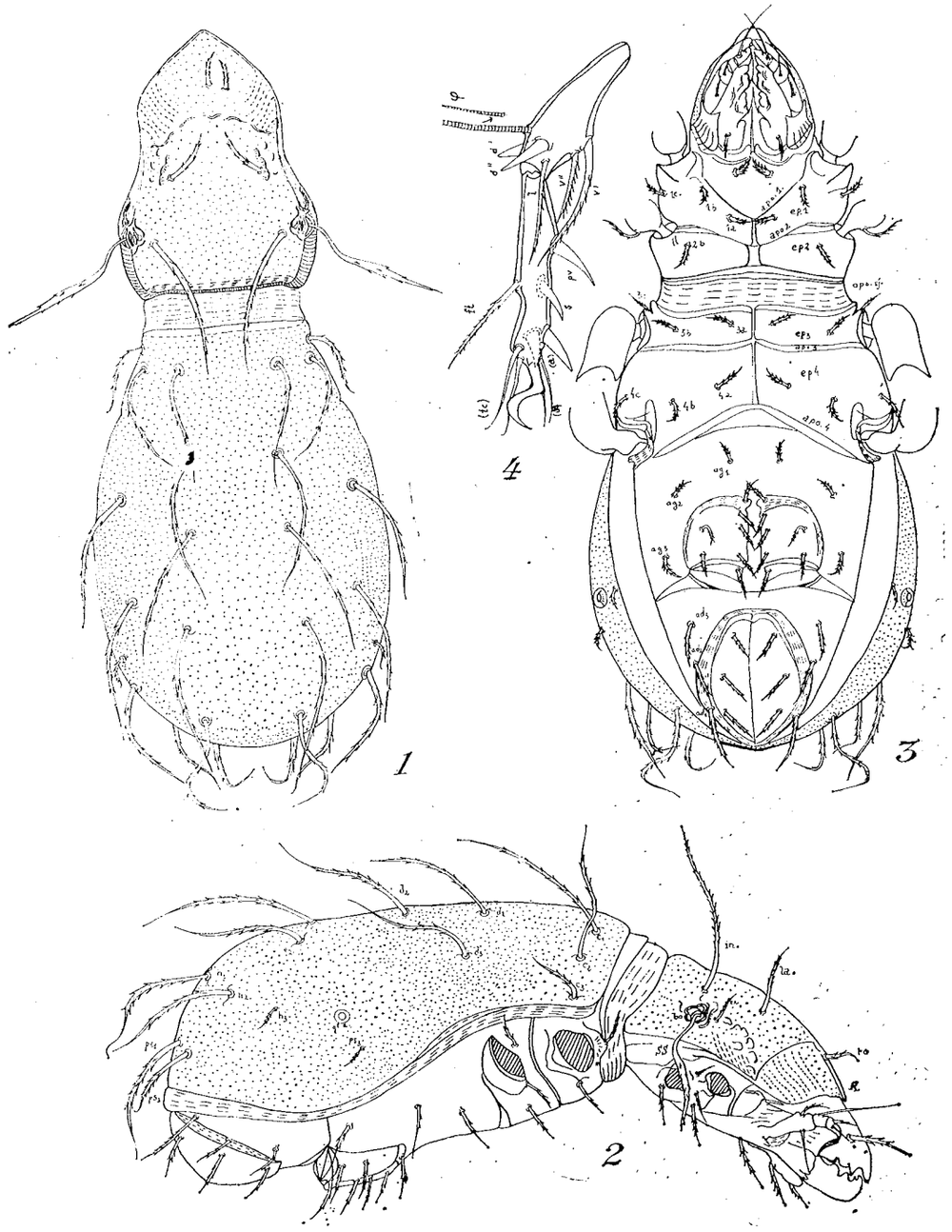
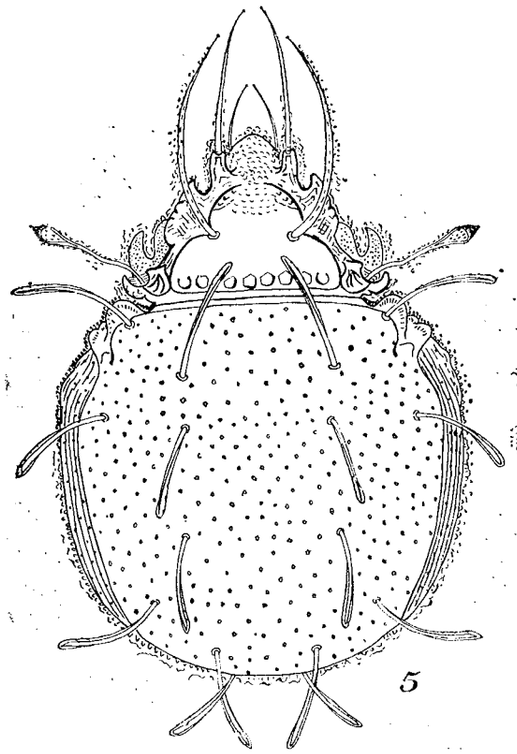


Fig. 1. — *Epilohmannia schusteri* — corps, vue dorsale.
Fig. 2. — *Epilohmannia schusteri* — corps, vue latérale.
Fig. 3. — *Epilohmannia schusteri* — corps, vue ventrale.
Fig. 4. — *Epilohmannia schusteri* — patte IV : le tarse et le tibia.



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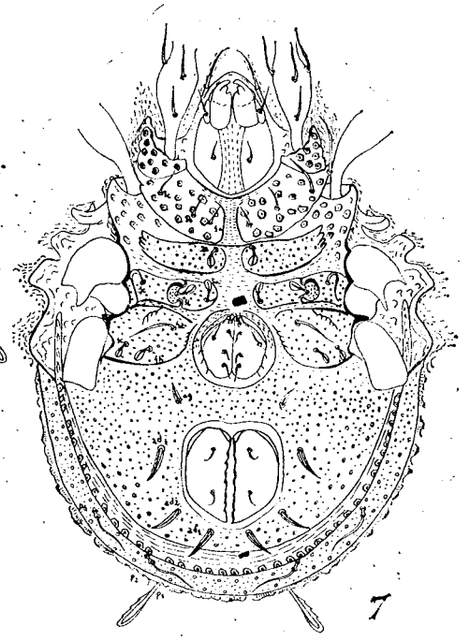

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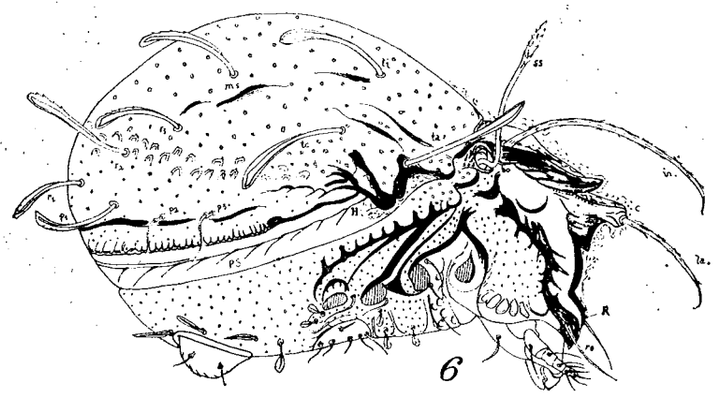

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Fig. 5. — *Hypocepheus krivolutskyi* — corps, vue dorsale.
Fig. 6. — *Hypocepheus krivolutskyi* — corps, vue latérale.
Fig. 7. — *Hypocepheus krivolutskyi* — corps, vue ventrale.

rieurement, huit épaississements chitineux polygonaux, disposés sur une seule rangée. Le notogaster nodohumerat, arrondi, a le bord antérieur droit et transversal et les bords latéraux fortement chitinisés. Parmi les dix paires de poils notogastraux, huit sont très longues, rigides et peu courbées et s'élargissent dans leurs parties terminales par des couvertures persistantes du tectostracum ; elles ont une position dorsale. Les deux paires de poils notogastraux (ps_2 et ps_3), disposées sur les parties latéro-postérieures du notogaster, sont fines, courtes et ne présentent pas de couvertures de tectostracum. Le notogaster, ornementé avec de petites fossettes arrondies, est recouvert de fines exfoliations du tectostracum (fig. 5).

Sur la face latérale, on observe les impressions musculaires qui sont disposées sur une rangée dorsale et sur une rangée située près du pli de déhiscence (fig. 6).

Sur la face ventrale, on observe le tutorium et le pedotectum 1 développés et pourvus d'ornementations polygonales. Le pedotectum 2 et le discidium sont étroits. Les poils épiméraux sont disposés d'après la formule : 3—1—3—3. Les volets génitaux entourés d'un anneau périgénital ont six paires de poils très fins, courts et lisses. Les poils agénitaux sont petits et pourvus de couvertures de tectostracum. Les volets anaux, éntourés d'un anneau périanal étroit, présentent deux paires de poils courts, fins et lisses. Les trois paires du poils adanaux ont aussi des couvertures de tectostracum. L'opistosoma présente une ornementation en forme de fossettes arrondies (fig. 7).

Les pattes monodactyles.

Holotype : adulte et sept paratypes chez les auteurs. Terra typica : bois de hêtre, Poieni (dép. de Jassy). Biotope : troncs pourris. Date : le 25 août 1974. Autres localités : le bois de hêtre Pîrcovaci, situé dans la région des sources de la rivière de Bahlui (dép. de Jassy), dans les troncs d'arbres pourris, le 9 septembre 1973, cinq exemplaires [2] [4].

L = longueur ; l — largeur.

BIBLIOGRAPHIE

1. Aoki J., 1965, Pacific Insects, **7**, *2*, 309—315.
2. Balogh J., 1972, *The Oribatid genera of the world*, Akad. Kiado, Budapest.
3. Fujikawa T., 1972, Insecta Matsumarana Journal of the Faculty of Agriculture Hokkaido University, **35**, *2*, 127—183.
4. Ghilearova M. S., Krivolutsky D. A., 1975, *Opredeliteli obitaiuscih v pocive klescei sarcoptiformes*, Akad. nauk S.S.R.
5. Schuster R., 1960, Zool. Anz., **165**, *5—6*, 197—213.
6. Walwork J. A., 1962, Acarologia, **4**, 670—693.

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MICROELECTRONOGRAPHY — A METHOD OF CELL RESEARCH

BY

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A new possibility of functional investigation of the cells, namely microelectronography is presented.
This method consists in obtaining some direct magnified images, or after autographic films performed through the luminiscence effect in conditions of exposing the living structures in the electric field, generated by high voltage currents.
The preliminary results obtained by investigating the vegetable structures and the human and bovine seminal liquid are presented.

The living organism exposed to high voltage and high frequency electric fields, under a certain critical tension regimen (near the piercing tension), shows luminous radiations of the "Corona effect" type at the level of the cover structures [1].

This phenomenon has been pointed out by Kirlian and utilized in the microscopic research of the living organisms [2].

The structures investigated this way are surrounded by a luminous hallo called "aura"; inside them, luminous or dark zones and points can be separated which reflect different functionalities [3].

The color spectrum of the luminous radiation differs from one organism to the other and even within the same organism [4].

The Kirlian phenomenon has been explained by the release of light quanta at the piercing limits of the dielectric by the field electrons; these piercings are determined by the polarization of the insulation dielectric with electric charges generated by the living organisms [5].

The luminous phenomena produced or projected on the photosensitive emulsion or on the surface of a photosensitive material produce an image that can be reproduced optically or electronoptically. The color differences in the light emission (blue, yellow, red) have been explained by the ionic structure of the biologic medium or of the gaseous cover surrounding it [6].

Starting from the Kirlian method, we have achieved and experimented an original method of investigating the living organism by placing it into a linear, direct, unidirectional electron accelerator; characteristic luminous effects are thus obtained which are controlled by the field parameters and by differentiation systems of the power value of the luminous emission [7]. The organic structures and their biofield may thus be separately differentiated (Fig. 1).

Unlike the Kirlian effect obtained under a regimen of radiofrequency (5.10^5—3.10^6Hz), under which the images appear compound, the image obtained through our method is fundamental.

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Under these circumstances the "aura" does not appear any longer being replaced by characteristic micropiercings differently oriented according to the sense of the impulse polarity used (Fig. 2).

The utilization of some filtering systems permits the power differentiation of the image. This method was applied to the macroscopic research of the organisms especially to pointing out the peculiar electronic behaviour of the malignant tumors [8].

Starting from these data, we applied the electronographic method to the research of the living microstructures (microelectronography).

METHODS AND TECHNIQUES

Two methods were used:

1. The *electronographic method* which is based on the microscopic photography of the images: the tissue to be examined is put into an electronographic device consisting of two metal leaves disposed on a glass slide and forming an insulation condenser on which the current is applied at the optimal parameters. The device is placed on a stage of a MC 1 IOR microscope, on its optic axis and the image is obtained by photography.

After regulating the image, the light source of the microscope is switched off and the source of high voltage current is released which is produced by an apparatus of the type "Electronograph 1" or "Electronograph 2" executed after Romanian patents [9] [10]. Common microscopic images can be obtained this way, as well as electronographic ones, that can be compared to the latter.

This method has the disadvantage of retaining some of the components of the light radiation emitted (the electronographic image consisting to a high degree in ultraviolet radiations). The deficiency of this technique has been avoided by utilizing the second method:

2. The *electronoautographic method* consists in obtaining images on photosensitive emulsions which are applied directly on the structures to be examined; these latter are electronographically exposed and then observed through light microscopes.

The exposures were carried out with the same types of electronographic apparatuses by unique and repeated releases.

ORWO RS 2 films sensitive to ultraviolet radiations, reversible AGFACHROM 50 S professional 120 films and special emulsions for tissular autographs, intended to radioisotope investigations carried out by the Institute of Atomic Physics, Bucharest, were used on this purpose.

The preliminary observations we are presenting have been achieved through the above mentioned methods in vegetable tissues and in human and bovine seminal liquids.

RESULTS

1. The presence of an electronographic image at the tissular and the cellular level could be noticed thus proving that the luminous emission is produced not only macroscopically, but also in the internal structures, as well as in the micro- and infrastructures (Figs 3 and 4).

2. The zones with maximum electric and energetic activity mark themselves out by the most luminous images clearly differentiated from the rest of the structures. Thus the cell membranes on the preparations of folios of *Allium cepa* bulb appear electronographically intensely lighted with a violet and ultraviolet spectrum band, thus demonstrating the role of cell membranes in electronogenesis (Figs 5—7).

Injured or necrotizing cells release red radiations thus differentiating from the ultraviolet emission of the cells with an intensive electric activity.

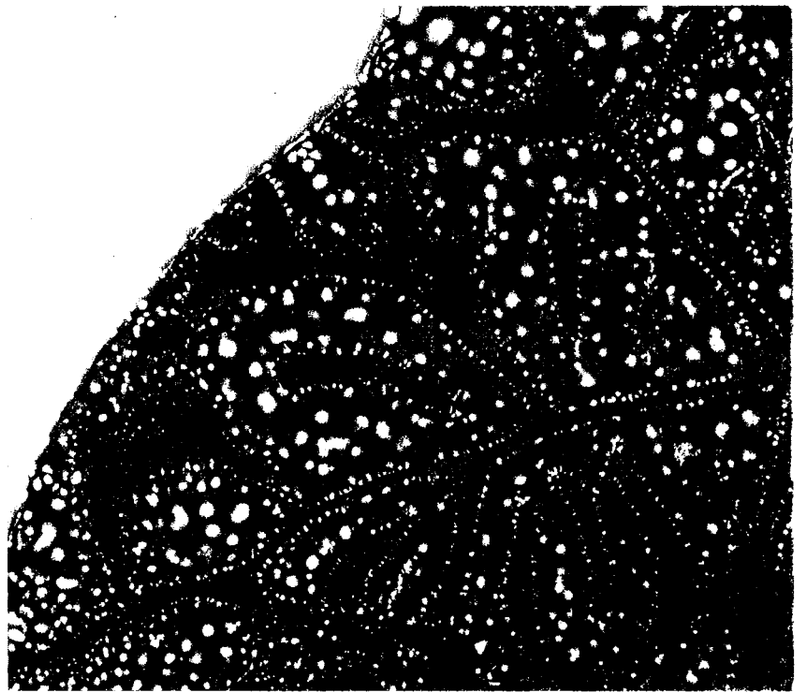

Fig. 2. - Vegetable macrostructure. Internal aspect.

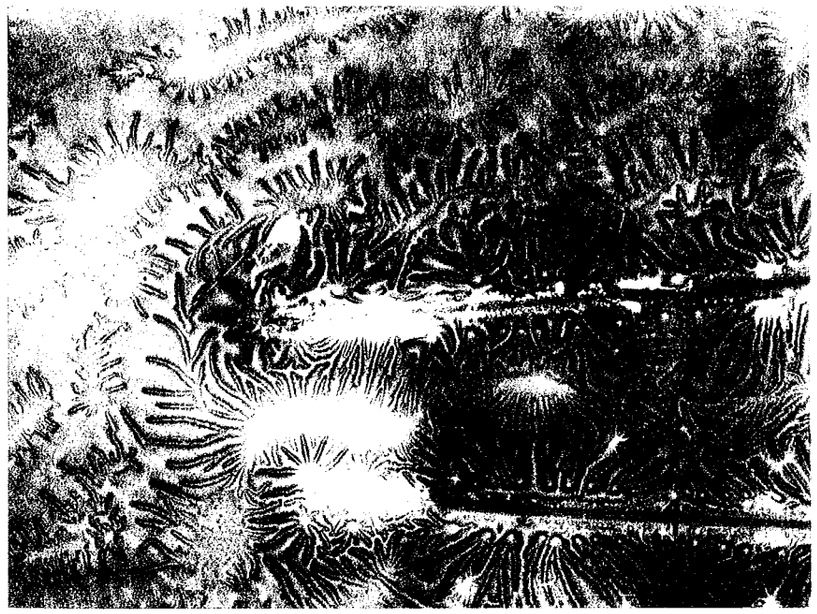

Fig. 1. — Electronography of two viral cultures (biofield effects).

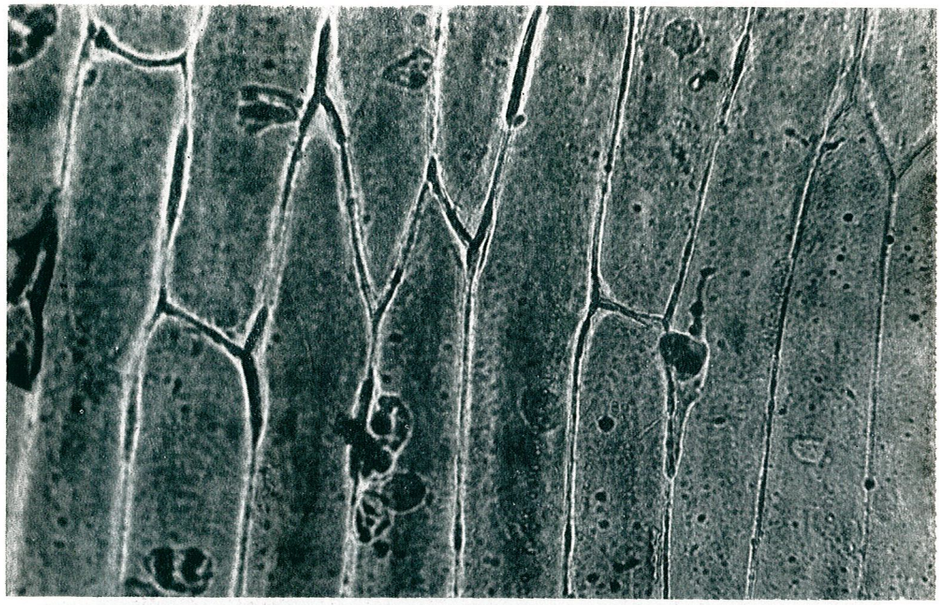

Fig. 3. — Microscopic normal photography of folios of *Allium cepa*.

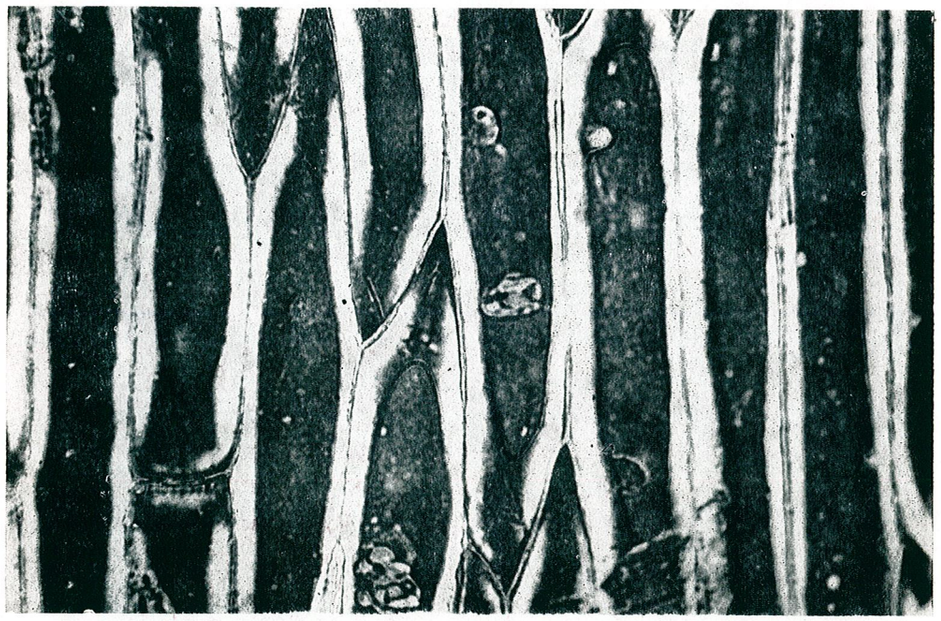

Fig. 4. — Microelectronography (autographic method) of folios of *Allium cepa*.

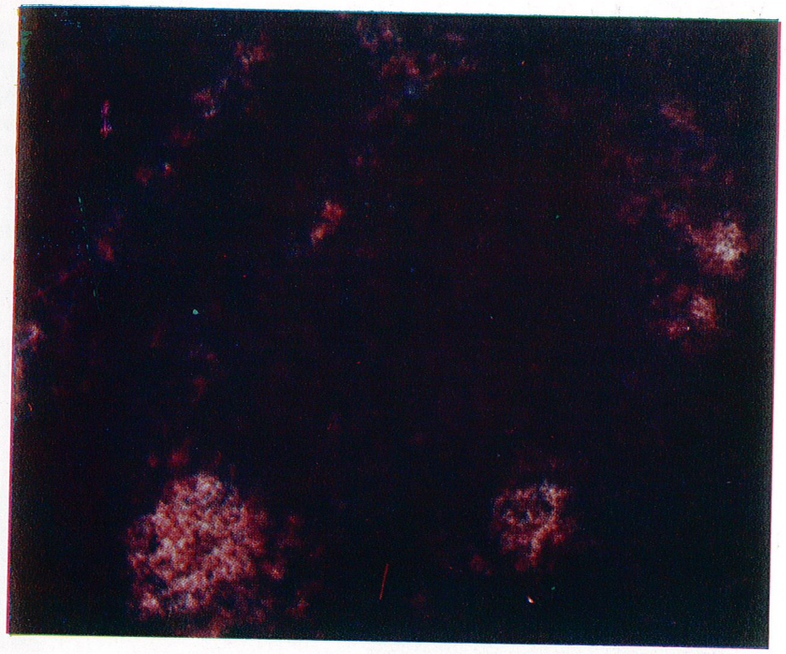

Fig. 5. — Color photographs of folios of *Allium cepa*.

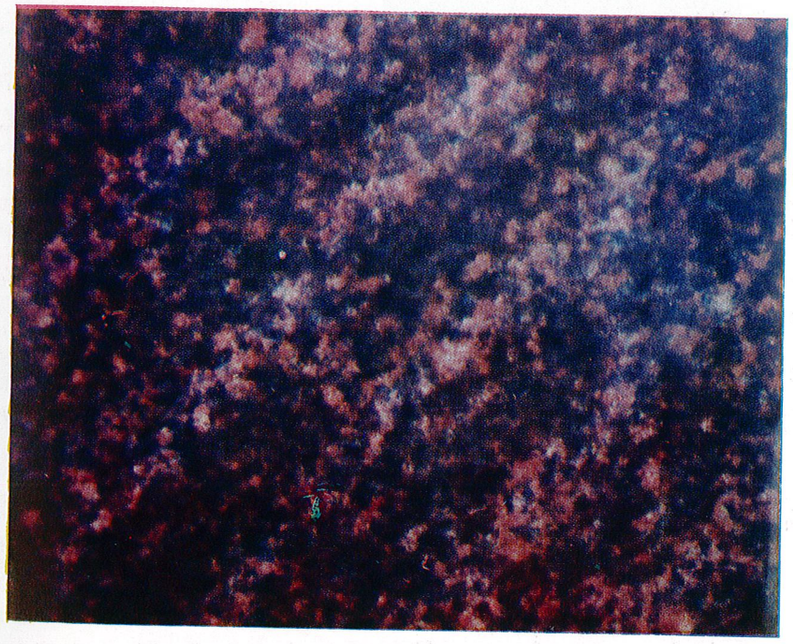

Fig. 6. — Different activities of cell membranes.

Fig. 7. — Electronography of a human spermatozoon.

3. The electric activity of the various cells making up a tissue appears electronographically differentiated. Thus some cell membranes prove to be inactive.

4. The microstructures with important energetic functions (e.g. the centrosome and the mitochondrial system occurring in the spermatozoon neck) are evidenced by a luminous sphere which appears separated from the spermatozoon's head. Inside the spermatozoal head, microstructures — differing from each other as to the electric polarity and intensity of the luminous emission — are also pointed out (Figs 8 and 9).

5. By means of the electronographic method, electric peculiarities can be emphasized which have no anatomic equivalent (e.g. the transversal striations on the spermatozoal tail or the zones of electric intercellular exchange — the electric pores of the cells).

DISCUSSIONS

By the observations and images presented above, a new method of cell exploring is discussed, which consists in obtaining the internal electric configuration by exposing this latter in high voltage fields generated by controlled impulses (following the original method) or in high voltage and frequency (according to Kirlian's method).

The extremely diversified domain of interest of macroelectronography does not allow us to point out in the present paper all the peculiar morphofunctional aspects characterizing the various types of cells and tissues.

We should like to assert that the research of the cell by means of the electroluminiscent phenomena is a modality of performing precise and reproducible exploration contrarily to the opinion according to which these phenomena would be generated only by the tissular and organic macropotentials.

An essential condition for carrying out this research is the exposure in monocellular layers and the uniform repartition of the electromagnetic field for each cell occurring in the acceleration space.

CONCLUSIONS

The electroluminiscence achieved by exposing living organisms in high-voltage electromagnetic fields represents an infrastructural phenomenon and allows morphofunctional correlations during the investigation of the cell.

REFERENCES

1. Greenler R. G., Mallmann A. J., 1972, Science **176**, 128—131.
2. Iniusin V. M., 1968, *On the Biological Essence of the Kirlian Effect* Alma-Ata, Kazakh U.S.S.R.-Kazakh State University.
3. Kirlian S. D., Kirlian V., 1961, J. Sci. Appl. Photogr. *6*, 397—403.

4. KIRLIAN S. D., KIRLIAN V., 1972, *Photography by means of high-frequency currents Dig. of the I-st Western Hemisphere Conf. on Kirlian Photography, Acupuncture and Human Aura.* N. Y.
5. ADAMENKO V. G., 1970, J. Paraphysics, *4*; 113–120.
6. TILLER W., 1972, *Some Comments on Kirlian Effect. Dig. of the 1st Western Hemisphere Conf. on Kirlian Photography Acupuncture and Human Aura*, N. Y.
7. DUMITRESCU I. FL., CELAN E., 1975, *Romanian Patent 82, 222*, O.S.I.M. Bucharest.
8. DUMITRESCU I. FL., GOLOVANOV N., CELAN E., GOLOVANOV, C., 1976, Cancer Res. Rev.
9. DUMITRESCU I. FL., NIȚULESCU H., 1975, *Romanian Patent, 82, 429* O.S.I.M., Bucharest.
10. DUMITRESCU I. FL., GOLOVANOV N., GOLOVANOV C., CELAN E., 1975, *Romanian Patent, 82, 556* O.S.I.M., Bucharest.
11. DUMITRESCU I. FL., 1975, *Omul și mediul electric.* Ed. Științifică, Bucharest.

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THE IONIC THEORY AND DONNAN EQUILIBRIUM OF THE MEMBRANE

BY

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The system intracellular fluid-membrane-extracellular fluid is, even in the simplest representation of the membrane, much more complex so that the membrane potential can not be conceived as an electrochemical potential of the Donnan type, as the ionic theory proposed at first. We present experimental and theoretical arguments which invalidate this kind of representation of the membrane potential, from which the ionists still keep important elements even in the present.

In the earliest days of its development, and we might say in its first approximation, the ionic theory on the biopotentials [5] [6] invoked for the explanation of the membrane potential the rules (equations) of the Donnan equilibrium.

Immediately after the excitable fiber membrane was proved to be permeable also for Na^+ ions [4] [9] [10] [12], the authors of the ionic theory gave up this explanation, considering the membrane potential as a result of the ionic diffusions permanently maintained by the pumps in both directions.

In connection with the position of the ionic theory regarding the Donnan membrane equilibrium, we would like to make some critical remarks, very important for the development of a more accurate point of view on the bioelectrical phenomena taking place at the level of biological membrane.

First of all, it should be pointed out that even in its first approximation (and in the conditions of the given model) the authors neglected some important aspects of the phenomenon which led them to some ambiguous conclusions that offered an incomplete or even distorted picture of the system and the discussed processes.

In the second place, we considered worth to make this analysis since with all the renewals brought into this concept after the presence and the activity of Na^+ and K^+ pumps were proved, the authors did not abandon some points of view adopted in the beginning, but tried to find new evidence for it.

There is no doubt about the fact that immediately after the unequal distribution of various ions on the two sides of the membrane was established, the attempt to find out if this could be explained on the basis of the Donnan membrane equilibrium was fully justified. But the analysis made by the ionists [2] [7] in this respect was in many ways deficient.

Admitting that the membrane is impermeable for big internal anions (A^-) and also for Na^+, it has been shown that the ratio of external : internal

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concentrations of Cl^- was equal to that of internal : external concentrations of K^+, that is in agreement with an equilibrium of the Donnan type :

$$\frac{[K^+]_i}{[K^+]_e} = \frac{[Cl^-]_e}{[Cl^-]_i}.$$

K^+ and Cl^- distributions in the two phases would thus result from a passive thermodynamic equilibrium : a perfect compensation between the diffusion forces (gradients of concentration) and the electrical ones. As a result of this approach, it was considered that the resting potential value of -90 mV (for the striated muscular fiber), measured experimentally, was in good agreement with the value calculated on the basis of the Nernst equation, which takes into consideration the internal and external concentrations of K^+ :

$$E = \frac{RT}{F} \ln \frac{[K^+]_e}{[K^+]_i} = -95 \text{ mV}.$$

On this basis, the idea that the membrane behaved as a K^+-electrode in resting state has been put forward. The positive charge of the external surface of the membrane is given by K^+ and the negative charge of the internal surface by the non-diffusible internal A^-. Thus, we would like to make the following observations to this conclusion (keeping general conditions admitted by the authors).

If the resting potential of the membrane is considered as essentially determined by K^+ (that is, as resulting from the ratio of external : internal concentrations of K^+ and from the fact that this latter diffuses to the outside through the membrane leaving in the intracellular compartment — or more exactly on the internal surface of the membrane — an equal number of negative charges represented by internal nondiffusible anions), it means that one has to take into account only a Donnan equilibrium through the membrane, unilaterally determined by the presence of big nondiffusible anions in the intracellular fluid. Of course, such an equilibrium (determined only by the internal nondiffusible charges) could be discussed only when the external phase did not contain any nondiffusible ions (as the Na^+ ions have been then thought). But in such a case (both Na^+ and K^+, being thought diffusible ions), a different distribution of Na^+ ions on one and another side of the membrane should be proved, than that established on an experimental basis.

It should have been :

$$\frac{[Na^+]_e}{[Na^+]_i} = \frac{[K^+]_e}{[K^+]_i},$$

which does not correspond with the experimental data. On the other hand, in the case considered as real at the time (nondiffusible Na^+), one should also have taken into account the part played by the extracellular Na^+ ions in determination of the positive electrical charge of the

external surface of the membrane, charge which could not have been ascribed only to K^+ ; and the same for Cl^- contribution to the negative charge of the internal surface of the membrane.

In the conditions admitted by the cited authors (nondiffusible Na^+), it would have been more accurate to consider the membrane potential as a complex result of two opposite Donnan equilibria, one imposed by the negative internal nondiffusible charges (A^-) and the other by positive external nondiffusible ones.

Therefore, even for the system considered by the authors (in the first days of ionic concept development), a membrane potential generated only by K^+ (and by A^- respectively) could not have been admitted, even though it has been stated that a "relatively good" agreement between experimental and calculated data was found.

Otherwise, we also consider the idea that the negative charge of the internal surface of the membrane would be determined only by the big internal nondiffusible anions as lacking experimental support.

In their experiments on giant perfused axons, Baker, Hodgkin., Shaw [1] showed that axons maintained in a normal external medium and perfused with isotonic KCl had a normal value of the membrane potential and also gave action potentials of normal amplitude and time course. (The same took place when other K^+ salts, as sulfate, methylsulfate, isothianate have been used.)

We are not going to discuss the relative role of certain internal anions for the maintainance of the membrane excitability, since the experiments of the cited authors have not been conducted in conditions as to point out this fact. What seems to be worth noticing is the evidence that the charge of the internal surface of the membrane could be as well achieved by the presence of small anions, in the intracellular medium, as Cl^- ions are, for which it has been shown that the membrane had a good permeability. We might recall that the radius of the hydrated Cl^- ion is equal with that of the hydrated K^+ ion [11] and that their mobilities are almost identical [3] [8].

It should be mentioned that neither the equation :

$$\frac{[K^+]_e}{[K^+]_i} = \frac{[Cl^-]_i}{[Cl^-]_e},$$

considered as demonstrating the appliability of the Donnan equilibrium to the biological membrane, did not have a reliable experimental basis. It has not been accurately assessed what was the real concentration of intracellular Cl^-, and the value admitted for this latter, was inferred on the basis of the experimentally recorded value of the membrane potential (-90 mV) and of the external Cl^- concentration, using the Nernst equation. But inferring by computation the value of an internal concentration of Cl^-, for its distribution to be in equilibrium with the real membrane potential (taking into account the external concentration of Cl^-), and then considering that Cl^- is actually in a passive thermodynamic equilibrium (therefore has this internal concentrations) does not mean that one made a proper demonstration.

It becomes clear that a unilateral determination of the biological membrane potential only by A^- and K^+ distributions on one side and the other of the membrane can not be admitted, nor even for the case when Na^+ was considered as nondiffusible. Thus the use of the Nernst equation, which takes into account only the internal and external concentrations of K^+, is not justified.

REFERENCES

1. Baker P. F., Hodgkin A. L., Shaw T. I., 1961, Nature (London), **90**, 885.
2. Boyle P. J., Conway E. J., 1941, J. Physiol. (London), **100**, 1.
3. Conway B. E., 1952, *Electrochemical data*, Elsevier Publishing, Company, Amsterdam.
4. Harris E. J., Burn G. B., 1949, Trans. Faraday Soc., **45**, 508.
5. Hodgkin A. L., Huxley A. F., Katz B., 1949, Arch. Sci. Physiol., **3**, 129.
6. Hodgkin A. L., Huxley A. F., Katz B., 1952, J. Physiol. (London), **116**, 424.
7. Hodgkin A. L., Horowicz P., 1959, J. Physiol. (London), **148**, 127.
8. Katz B., 1966, *Nerve, Muscle and Synapse*, Mc. Graw-Hill Book Comp., New York.
9. Keynes R. D., 1954, Proc. Roy. Soc. (London), **142**, 359.
10. Levi H., Ussing H. H., 1948, Acta Physiol. Scand., **16**. 232.
11. Shanes A., 1958, Pharmacol. Rev., **10**, 59.
12. Steinbach H. B., Spiegelman S., 1943, J. cell. comp. Physiol., **22**, 187.

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THE DONNAN EQUILIBRIUM AND THE NERNST EQUATION FOR THE CONCENTRATION CELL

BY

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Although after the discovery of the membrane permeability to Na^+, the ionic theory gave up the idea that across the biological membrane an equilibrium of the Donnan type was established, important elements of such a way of dealing with the membrane potential were kept on unjustifiedly and did not abandon the use of the wellknown equation (Nernst equation) which is valid only in the case of a Donnan equilibrium. The authors bring experimental and theoretical evidence in this respect.

The idea of biological membrane impermeability to Na^+ was abandoned after the experiments using radioactive tracers have proved the existence of a permanent Na^+ inflow across the membrane in resting state [5] [9] [10] [12] etc.

The experiments with radioactive compounds have also shown that Na^+ inflow was accompanied by an approximately equal K^+ outflow. It was admitted that Na^+ and K^+ pumps were maintaining the unequal distribution of the ions on one and the other side of the membrane by an "uphill transport", based on a metabolic energy consumption. Therefore, the active maintenance of specific ionic concentration gradients across the membrane would also explain the constant value of the membrane potential.

Nevertheless, in these conditions, the idea that the K^+ distribution would result from a passive thermodynamic equilibrium was abandoned. It is particularly important to point out that K^+ is in such an equilibrium, that there is no perfect correspondence between the calculated value (by Nernst equation) and that experimentally measured of the resting potential. Thus, the idea that the membrane potential is actually a diffusion potential continuously maintained by the pumps, the value of which being determined, on the one hand, by the ratio between the Na^+ and K^+ membrane permeabilities (in resting state the membrane is about 50 to 75 times more permeable to K^+ than to Na^+) and, on the other hand, by the pumping rate of these ions [8] etc., was forwarded. In other words, the idea of a Donnan equilibrium was abandoned. However, the idea that in a stady state the Cl^- ions are distributed in equilibrium with the membrane potential was maintained because, in fact, no experimental proof pleading for an active Cl^- transport could be found for the neural or muscular fibers [1] [6] etc. Also the following relation :

$$E = \frac{RT}{F} \ln \frac{[K^+]_e}{[K^+]_i}$$

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for the membrane potential calculation, was maintained being considered as an equation giving a fairly good value [7]. Some attempts for its improvement have been made, using so called "constant field" equation formulated by Goldman [3]. Among these equations we recall :

$$E_m = 58 \log \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_e}{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i}$$

or

$$E_m = \frac{RT}{F} \ln \frac{[K]_e + b[Na]_e}{[K]_i + b[Na]_i},$$

where

$$b = \frac{P_{Na}}{P_K}$$

(Hodgkin, [6]).

But the results were not satisfactory enough being considered finally only as semi-quantitative means to estimate the membrane potential value [11].

This failure arised because the "constant field" equation admitted too many simplifying suppositions regarding the membrane. This latter was considered as having a perfect homogenous structure and a perfectly uniform internal electrical field, the ions moving according to both concentration and potential differences, like in a free solution (independently ones from anothers). At the interfaces contacting the intracellular and extracellular phases, respectively, the ionic concentrations would be determined by the distribution coefficients, that is directly proportionally with the concentrations in the adjacent aqueous solutions. The permeabilities to ions, which are proportional to the mobilities and ion distribution coefficients, would be unaffected by ions or by the membrane potential and no interaction would exist between ions moving in opposite directions or between different species of ions.

As regards the use of the Nernst equation :

$$E_m = \frac{RT}{F} \ln \frac{[K^+]_e}{[K^+]_i}$$

as a direct relation for the membrane potential calculation or as a basis for the other relations mentioned above, we would like to show that this was justified particularly by the idea that through the membrane a Donnan equilibrium would be reached.

In fact, this is the equation for the calculation of electromotive force (e.m.f.) of a concentration cell which is function of the difference between the potentials of the two cell electrodes :

$$E_{conc.} = e_1 - e_2 = \left(e_1^0 + \frac{RT}{nF} \ln C_1\right) - \left(e_2^0 + \frac{RT}{nF} \ln C_2\right).$$

The two electrodes of the concentration cell are, in our case, the silver chloride electrodes of the measuring system. As $e_1^0 = e_2^0$, the equation can be simplified to :

$$E_{conc.} = \frac{RT}{nF} \ln \frac{C_1}{C_2}.$$

This relation, as written above, does not take into account the phenomena at the separation limit between the two solutions with different concentrations of the same electrolyte, but only those occurring at the electrodes. The relation completion to express the whole (entire) e.m.f. of the cell depends, however, on the nature of the concentration cell.

In the case of a membrane through which a Donnan equilibrium was reached, at the separation limit of semi-cells the value of the electrochemical membrane potential was equal and of opposite sign with the potential difference of the concentration cell formed by inserting identical reversible electrodes in the two compartments. Thus, it is not necessary in this case to complete effectively the relation, but evidently, the experimental result of the measurement should be correctly interpreted taking into account the specific conditions mentioned above. If this was the case with the Nernst equation, the fact that this latter was still used even after the ionists gave up the idea that a Donnan equilibrium was reached through the biological membrane is surprizing. This is even more surprizing as the inapplicability of this equation for the biological membrane potential calculation could have been easily found out, even before it was proved that the resting potential was not a Donnan electrochemical potential. When a given membrane reaches such an equilibrium between the phases, the membrane electrochemical potential being equal and of opposite sign with the concentration cell potential, is not apparently manifest, and the recording system should indicate a potential difference equal to zero. The value of a Donnan membrane potential is measured either by a sudden removal of the membrane from the system measuring in these conditions the concentration cell potential (that is not possible for the biological membrane) or by the determination of the e.m.f. of the concentration cell in some other way (for instance by individual measurings of each electrode potential or by the calculation of e.m.f. of the concentration cell based only on the electrode potentials in known internal and external K^+ concentration) [2] [4] [13]. But the measurements of the biological membrane potential are made in conditions when, if one would deal with a Donnan potential, a zero potential should be recorded with the measuring device, and in fact, a recorded value of −90 mV was obtained.

Therefore, the equation used by the ionists for the membrane potential calculation is, as a rule, valid for a membrane through which a Donnan equilibrium was reached, but the experimental data obtained by measurements on the biological membrane refute in this case the validity of the relation.

It is true that the e.m.f. of a concentration cell, according to the relation :

$$E_c = \frac{RT}{nF} \ln \frac{C_1}{C_2} \neq 0$$

depends on the ratio of diffusible ion concentrations in the two phases (and in fact only on the concentration ratio, no matter their absolute values). But, such a simple interdependence assumed that only the phenomena taking place at the electrodes (measuring electrodes) should be taken into account and not also those occurring at the separation "limit" between the two phases. When such a close experimental confirmation of this relation will be found by potential recording on the biological membrane, it would mean that the separation limit of the phases, namely the membrane, would not contribute, from an electrical point of view, to it.

The membrane electrical potential would be practically null and would not affect the potential difference between the two recording electrodes (that is, in resting state, the membrane would be lacking a potential). Such a conclusion has no support.

Therefore, since the biological membrane does not achieve an electrochemical potential of the Donnan type (also obvious in the experimental recording of the potential), the use of the Nernst equation by the ionists, discussed above, for the calculation of the membrane potential, is neither theoretically justified, nor experimentally supported.

Since the ionists made no substantial attempt to overcome their own membrane model and, at the same time remained tributary to the way to conceive the generation of the membrane potential adopted in the first days of this concept development, we might state that the ionic theory strikes against its own limits.

REFERENCES

1. Conway E. J., 1957, Physiol. Rev., **37**, 84.
2. Erdey-Grúz T., Schay G., 1957, *Chimie fizică teoretică*, Ed. tehnică, Bucureşti.
3. Goldman D. E., 1943, J. Gen. Physiol., **27**, 37.
4. Grémy F., Pagès J.-C., 1966, *Biophysique*, Ed. médicales Flammarion, Paris.
5. Harris E. J., Burn G. B., 1949, Trans. Faraday Soc., **45**, 508.
6. Hodgkin A. L., 1958, Proc. Roy. Soc. (London), B., **148**, 1.
7. Hodgkin A. L., 1967, *The Conduction of the Nervous Impulse*, University Press, Liverpool.
8. Katz B., 1966, *Nerve, Muscle and Synapse*, Mc. Graw-Hill Book Comp., New York.
9. Keynes R. D., 1954, Proc. Roy. Soc. (London), B, **142**, 359.
10. Levi H., Ussing H. H., 1948, Acta Physiol. Scand., **16**, 232.
11. Shanes A. M., 1958, Pharmacol. Rev., **10**, 59.
12. Steinbach H. B., Spiegelman S., 1943, J. cell. comp. Physiol., **22**, 187.
13. Ţilenschi S., 1964, *Chimie coloidală*, Ed. Tehnică, Bucureşti.

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THYMUS AND BURSA FABRICII REACTIONS UNDER THE ACTION OF ACTH AND OF THE BERNARDI-COMŞA THYMUS HORMONE

BY

RODICA GIURGEA and VIRGIL TOMA*

In 45-day Studler-Cornish chickens, the ACTH-elicited involution of thymus and bursa can be reduced by concomitant administration of Bernardi-Comşa thymus hormone. This effect is manifested by significant increases of thymus and bursa total proteins, of bursa RNA and of thymus DNA.

The reversible involution of the thymus elicited in states of stress by the hypersecretion of adrenal hormones has been demonstrated by Selye, in 1936 [10]. More recently, an incretum of the thymus epithelium was told to induce the immunocompetence of thymocytes and to influence the homeostasis of the endocrine glands [2] [9]. Based on the cybernetic models in endocrinology constructed by Milcu [8], the problem may be put whether feedback relations do exist between the thymus secretion and the ACTH level. Aiming at answering this problem, we followed some modifications which occurred in the thymus and the bursa of young chickens, during the involution provoked by ACTH and during concomitant Bernardi-Comşa thymus hormone (BCTH) administration.

MATERIALS AND METHODS

Experiments were performed on Studler-Cornish chickens aged 45 days, which have been reared in standard conditions. Our experimental model included the following variants :

I. An ACTH treated group (ACTH-CIF, in daily i.m. doses of 2.5 IU/100 g during 3 days). This group was taken as control, having in view that the property of this hypophyseal hormone to elicit thymus and bursa involution is well known [3] [5].

II. A group treated with ACTH as I, but receiving in the first day also a single i.m. dosis of 130 μg BCTH/100 g.

III. A group treated with ACTH as I, but receiving daily i.m. doses of BCTH, 130 μg/100 g, during the three days.

IV. A group treated with daily i.m. doses of BCTH, 130 μg/100 g, during three days (without ACTH).

24 hours after finishing the treatments, animals were sacrificed by decapitation, thymus and bursa isolated and weighed on a torsion balance. DNA and RNA concentrations were determined by Spirin's spectrophotometric method [11] and the total protein content [TP] was assayed by the method of Robinson and Hogben, modified by Korpaczy (cited after [6]). Results were expressed as percentage differences against the control group [I]. The statistical significance of the differences was checked by Student's "t" test.

* With the technical assistance of St. Ilyes

RESULTS AND DISCUSSIONS

As shown in table 1, BCTH administered to young chickens (group IV) leads to a statistically significant increase of the thymus DNA (+84%, $p < 0.01$) and bursa RNA (+ 48%, $p < 0.001$). This fact may be put in relation with the peculiar role of the thymus in the synthesis and metabolism of the nucleic acids and proteins as shown by Milcu and Potop [9]; the amount of these substances is lowered in the liver after thymectomy and increased after thymus extract administration. On the other hand, the increase of thymus DNA under the action of BCTH suggests a stimulatory effect of this substance on the well known cellular immunitary function of this gland [2]. There are many data [7] [9], showing that the thymus, or its active principles, exert an inhibitory effect on carcinogenesis by an immunocellular way.

The thymus involution induced by ACTH is so highly specific, that it is used for testing the hormone [3]. In a previous paper, we have shown that in chickens both the involution and the regeneration of the thymus and bursa are coupled with specific modifications in the metabolism of the nucleic acids and proteins [5]. These modifications are determined by the ACTH-induced hypersecretion of glucocorticosteroids, the lymphocytes of the thymus and probably also of the bursa possessing receptors for these hormones [1].

Table 1

Modifications of DNA, RNA and total protein (TP) contents and thymus and bursa weights of chickens treated with ACTH and Bernardi-Comşa thymus hormone

Substance modified		I. ACTH (CONTROL)		II. ACTH-BCTH		III. ACTH—BCTH		IV. BCTH	
		Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa
DNA mg/g	Mean	8.32	3.30	7.56	2.55	17.53	4.62	15.35	4.10
	ES ±	1.03	0.95	1.00	0.67	0.75	0.53	1.58	0.59
	n	8	8	8	8	7	8	8	8
	± %	—	—	−10	−23	+110	+40	+84	+24
	p	—	—	—	—	<0.001	—	<0.01	—
RNA mg/g	Mean	10.11	3.89	4.27	15.36	7.36	5.11	7.37	5.78
	ES ±	1.76	0.15	0.48	1.01	0.19	0.32	0.45	0.53
	n	8	8	8	7	6	8	8	7
	± %	—	—	−58	+294	−28	+31	−28	+48
	p	—	—	<0.01	<0.001	—	<0.01	—	<0.001
TP mg %	Mean	23.04	16.86	14.35	15.93	32.25	26.66	30.99	14.01
	ES ±	5.34	1.16	0.70	1.74	5.60	6.24	8.16	1.39
	n	8	8	7	7	7	7	8	7
	± %	—	—	−38	−6	+39	+58	+34	−17
	p	—	—	—	—	<0.01	<0.01	—	—
Organ weight mg	Mean	1215	450	917	337	635	407	1172	308
	ES ±	194	43	90	58	70	59	31	33
	n	8	8	7	8	8	8	6	7
	± %	—	—	−25	−26	−48	−10	−4	−32
	p	—	—	—	—	<0.02	—	—	—

Our experiments with combined ACTH and BCTH treatment show an ability of the thymus principles to protect in a high degree both the thymus and bursa against the lysis elicited by ACTH or by endogeneous glucocorticosteroids.

The significant ($p < 0.01$) increases of TP both in the thymus (+ 39%) and the bursa (+ 58%), of RNA in the bursa (groups II and III) and of DNA in the thymus (group III, + 110%, $p < 0.001$) are indications in this direction.

Comşa [2] has shown a general immunosupressor action of ACTH, which accelerates the rejection of skin grafts; this effect is greatly reduced by thymus hormones. Our results mentioned above support and explain the data of Comşa, having in view that the involvement of nucleic acids in the immunological processes including the thymocytes is well established [4].

The enhancement by BCTH of the RNA reduction elicited in the thymus by ACTH (an effect opposed to that observed in the bursa) may be attributed to an inadequate dosis of the thymus principle.

Besides their immunological implications, our data suggest a reciprocal character of the antagonism between ACTH (respectively glucocorticosteroids) and the thymus. Such feed-back reactions may constitute means of correction of adrenal homeostasis. Transposing these data into the medical practice, a thymic therapy would be recommended to protect the immunobiological systems against the thymolysis of adrenal origin.

In conclusion, thymo-bursal involution elicited by ACTH administration in young chickens can be reduced through BCTH therapy, which leads to a significant increase of TP both in the thymus and the bursa, of RNA in the bursa and of DNA in the thymus.

REFERENCES

1. Abraham A., 1975, *Mecanismul de acţiune al hormonilor steroizi*. Bucharest, Ed. Academei.
2. Comşa J., Leonhardt H., Schwartz J. A., 1975, Ann. New York Acad. Sci., **249**, 387.
3. Dorfman R. I., 1962, *Methods in hormone research*. Acad. Press New York, London.
4. Friedmann H., 1973, Ann. New York Acad. Sci., **207**, 485.
5. Giurgea R., Toma V., 1975, Zbl. Vet. Med. A., **22**, 485—492.
6. Kovach A., 1958, *A kisérletes orvostudomany vizsgalo modszerei*. Akad. Kiado, Budapest, **2**, 28—29.
7. Metcalf D., 1966, *The thymus*. Springer Vrlg. Berlin, Heidelberg, New York.
8. Milcu St., M., 1965, Fiz. Norm. Pat. **11**, 413
9. Milcu St. M., Potop I., 1970, *Farmacodinamia substanţelor hormonale asemănătoare din timus*. Ed. Academiei, Bucharest.
10. Selye H., 1936, Brit. J. Exp. Path., **17**, 234—246.
11. Spirin A. S., 1958, Biohimia, **23**, 656.

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EVOLUTION OF ALKALINE AND ACID INORGANIC PYROPHOSPHATASE IN THE FIRST DEVELOPING STAGES IN HAPLOID AND DIPLOID EMBRYOS OF *RANA RIDIBUNDA*

BY

RADU MEȘTER and DRAGOȘ SCRIPCARIU

Acid and alkaline inorganic pyrophosphatase were determined in total proteic extracts of the following embryonic stages : egg, two-cells stage, blastula, gastrula and neurula. Comparative studies revealed that the enzymes are very much alike both in haploid and diploid embryos. Following the evolution of these enzymes in the first embryonic stages, we found that in haploid embryos their specific activities were by 50% lower than in diploid embryos.

The biosynthesis of nucleic acids, coenzymes, proteins and steroids shows one or more synthetases (pyrophosphorylases) releasing inorganic pyrophosphate. The accumulation of pyrophosphate in the cell determines the inhibition of biosynthesis metabolic processes. The coupling of these reactions with the hydrolysis of the simultaneously produced pyrophosphates shifts the general balance of the reactions to the benefit of synthesis. The fact that the pyrophosphate hydrolysis is favouring the RNA biosynthesis has been experimentally proved [9]. It was equally proved that the accumulation of pyrophosphate inhibits RNA *in vitro* [9] [14]. Alongside from pyrophosphate, also other factors (nature and concentration of ions, enzymes' concentration, a.s.o.) may interfere in the regulation of nucleic acids biosynthesis.

The study of the development of amphibian embryos with different ploidy has made the object of numerous investigations, chiefly in terms of cytology and morphology [3] [5—7]. Haploidy has, in the first place, a genetical basis, due to the presence, in the nucleus, of a set of haploid chromosomes.

Taking into account the relationship between the cellular genetical information and the enzymatic activity, on the one hand, and the impact of the inorganic pyrophosphatase phosphohydrolase on the biosynthetic mechanisms and on the cell primary energetic economy, on the other hand, we assumed the task of examining the enzymatic activity during the first development stages in diploid and haploid embryos of *Rana ridibunda*.

While extensively studied in plants and animals, mainly as to the distribution and the kinetical properties [2] [8] [11] [16], it is very little known in lower vertebrates [10] and almost at all in embryonic development. Beloff [1] made a histochemical study on inorganic pyrophosphatase in embryos of *Rana pipiens*. The author found a relatively uniform distribution of this enzyme in all organs investigated.

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MATERIAL AND METHODS

Rana ridibunda frogs obtained from the lakes around Bucharest have been used. The frogs ovulation was experimentally achieved in the laboratory, by administration of hypophyseal gonadotrophin (200 IU per frog), prolan and total proteic extract from the hypophysis of frogs. To obtain spermatozoa, the males were given injections with 100 IU of hypophyseal gonadotrophin.

Fertilization of the frog eggs was made artificially with sperm suspension. To obtain haploid embryos, the sperm suspension was irradiated before fertilization, in UV, with a HBC 200 source. The following embryonic stages have been studied: egg, two-cells stage, blastula, gastrula, neurula with neural groove, tadpole with tailed burgeon and swimming tadpole.

The embryonic forms of haploid and diploid embryos were released by mechanical discarding of the capsule. For each stage, total proteic extracts were obtained by homogenization of embryonic forms in a Potter homogenizer, with distilled water (10 embryonic forms per ml). The protein extraction was made at cold, for two hours. The supernatant obtained after centrifugation at 8000 r.p.m., for 15 min, was used for enzymatic determinations.

The activity of the inorganic pyrophosphatase was evidenced through the determination of the orthophosphate released upon the enzymatic hydrolysis of pyrophosphate, in acid or alkaline buffer solution, according to the method of Fiske-Subbarow [4]. The enzymatic activity was expressed in μ moles P_i per mg protein per min.

The protein concentration in the total proteic extracts was determined spectrophotometrically at 280 nm.

RESULTS

To assess the optimal enzyme activity, the effect of pH on the inorganic pyrophosphatase activity in the total proteic extracts of non-fertilized frog eggs was followed up. As shown in figure 1, the frog eggs contain two inorganic pyrophosphatases : one active in acid medium (optimal pH 3) and another one in alkaline medium (optimal pH 8). The inorganic acid pyrophosphatase is active in the absence of Mg^{2+} ions.

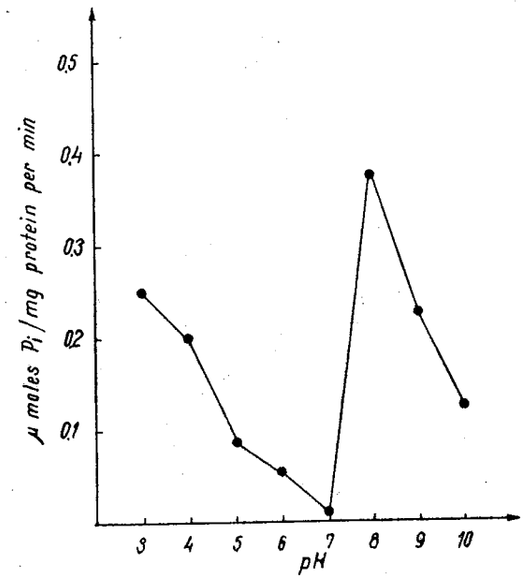

Fig. 1. — Effect of pH on the enzymatic activity of inorganic pyrophosphate phosphohydrolase of non-fertilized frog eggs.

The enzymatic activity of acid inorganic pyrophosphatase decreases as the magnesium ions concentration increases. Thus, at a 200 μ moles Mg^{2+} concentration, the activity of acid pyrophosphatase is inhibited by about 50% (Table 1).

The inorganic alkaline pyrophosphatase depends on the presence of Mg^{2+} ions. Noteworthy is the fact that the optimum concentration on Mg^{2+} ions of alkaline pyrophosphatase is 20 μ moles, a concentration which does not significantly influence the activity of acid form. Higher concentrations of magnesium ions do not considerably affect the activity of alkaline pyrophosphatase.

Table 1

The influence of Mg^{2+} ions on the activity of acid inorganic pyrophosphatase in the total proteic extract of frog eggs

Mg^{2+} (μmoles)	activity (%)
0	100
40	89
80	74
120	63
200	49

The activity of inorganic pyrophosphatase is proportional to the concentration in the substrate, within relatively large limits. The high concentrations in the substrate do not influence the enzymatic activity (Fig. 2). The maximal pyrophosphatase activity can be obtained with a Mg^{2+}/PP_i ratio of 1 : 1 up to 2 : 1.

The optimum concentration of substrate for alkaline and acid inorganic pyrophosphatase in haploid and diploid embryos of frogs shows that there are not two different enzymes. As it results from figure 2, the saturation curves with pyrophosphate of the alkaline pyrophosphatase (also valid for the acid one) are the same for both haploid and diploid embryos. Only the enzymatic activities differ, being higher in diploid

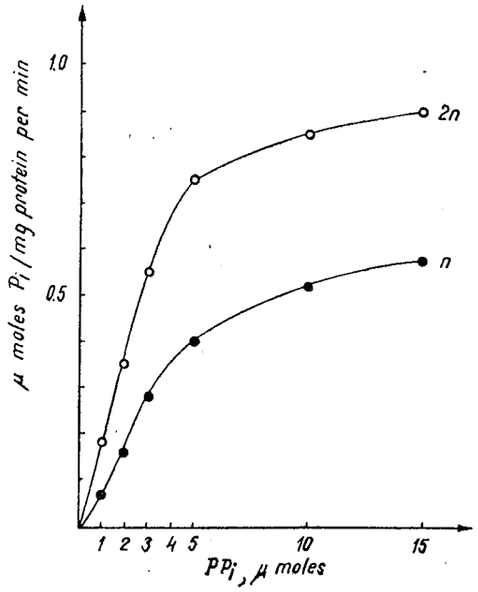

Fig. 2. — The influence of the substrate concentration upon the activity of alkaline inorganic pyrophosphatase from diploid (2n) and haploid (n) embryos of *Rana ridibunda*.

embryos. The Michaelis constants (Km) computed from the saturation curves are of 2.6 mM (n) and 2.9 mM (2n).

Evolution of the activity of alkaline inorganic pyrophosphatase in frogs embryos. As shown in figure 3, the non-fertilized eggs of *Rana ridibunda* show an alkaline pyrophosphatase activity of about 315 μmoles P_i per mg protein per min. In all stages that were examined, the haploid

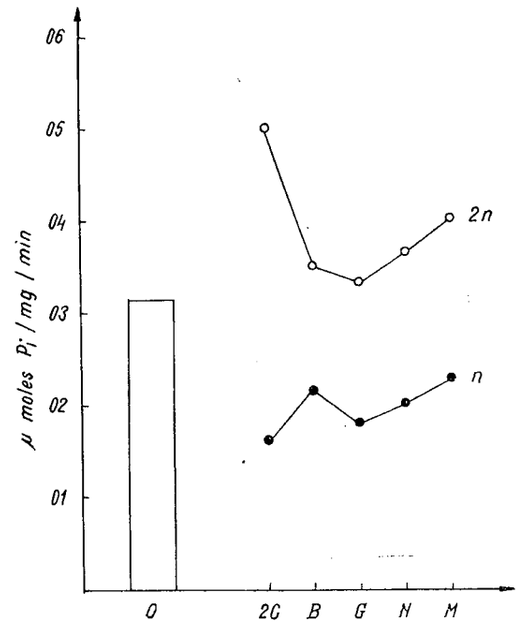

Fig. 3. — Evolution of the activity of alkaline inorganic pyrophosphatase in the first developing stages, in haploid (n) and diploid (2n) embryos of *Rana ridibunda*. *O* egg; *2C*, two-cells stage; *B*, blastula; *G*, gastrula; *N*. neurula; *M*, tadpole.

embryos had a decreased alkaline pyrophosphatase activity, ranging between 160 μmoles P_i per mg protein per min, in the two-cells stage, and 227 μ moles P_i per mg protein per min in the tadpole stage. In diploid embryos a wider fluctuation of the alkaline pyrophosphatase activity was found, namely : 500 μ moles P_i/mg protein per min in the two-cells stage, and 410 μmoles P_i/mg protein per min in the tadpole stage. The mean activity of alkaline inorganic pyrophosphatase for the 5 stages studied is about 400 μ moles P_i/mg protein per min in diploid embryos and about 200 μmoles P /mg protein per min in haploid embryos.

Figure 4 presents the evolution of the acid inorganic pyrophosphatase activity, both in non-fertilized eggs and in the first embryonic development stages in haploid and diploid embryos. A relatively significant acid pyrophosphatase activity was found in non-fertilized eggs (350 μmoles P_i/mg protein per min). Despite the wide fluctuations in the enzymatic activity within each stage and embryo type (haploid and diploid), the acid pyrophosphatase activity proved to be much higher in all stages examined in diploid embryos as compared with the enzymatic

activity in haploid embryos. The acid inorganic pyrophosphatase, computed per stages, is approximately by 38—50% lower in haploid embryos as against diploid embryos.

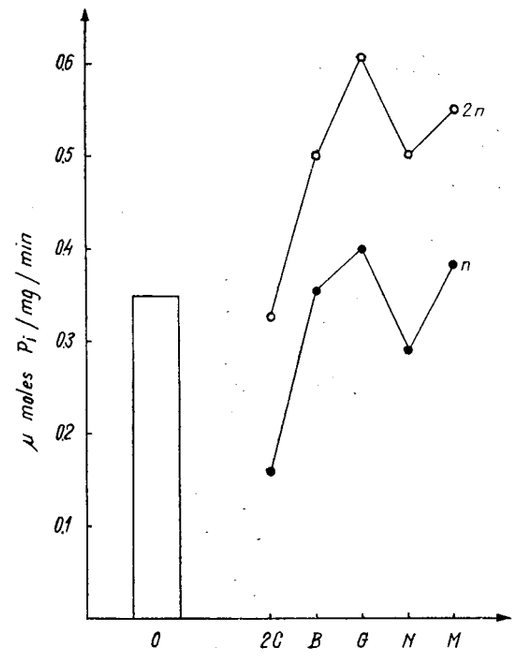

Fig. 4. — Evolution of the activity of acid inorganic pyrophosphatase in the first developing stages, in haploid (n) and diploid (2n) embryos of *Rana ridibunda*. Explication as in figure 3.

DISCUSSION

Histoenzymologic investigations, as well as numerous quantitative studies disclosed the participation of the enzymes in the course of the embryonic development and the cellular differentiation [13]. The development implies the presence in the eggs and in the embryos of some ubiquitous enzymes throughout the embryogenesis and of some specific enzymes that emerge or disappear during the different development stages.

The presence of both alkaline and acid inorganic pyrophosphatase in the non-fertilized eggs in *Rana ridibunda* shows their importance in the "phosphate cycle" of the cell as well as in the biosynthesis processes started after fertilization.

Following up the evolution or these enzymes in the first embryonic stages, just after fertilization, we found that in haploid embryos their specific activity was by 50% lower than in diploid embryos. The difference between the genetical sets 2n and n of diploid and haploid embryos accounts for the differences found in the specific enzymatic activity of inorganic pyrophosphatases. This entails secondary effects on numerous

enzymatic syntheses, implicitly the synthesis of all RNA types, of coenzymes, etc.

The cytological and electronmicroscopy studies carried out by Fox and Hamilton [5] revealed a delayed digestion of the vitellus and of the lipid drops in the haploid cells. The authors assumed that the energetics of the haploid cell is low, which results in a lower synthesis process of macromolecules and a deficient active transport at the membranes level. The fact that the specific activity of alkaline and acid pyrophosphatase is lower in haploid embryos than in non-fertilized eggs, suggests the importance of some repressive proteins and of other factors regulating the enzymatic activity, obviously modifying the functional activity of enzymes.

Our experimental data demonstrate that the synthesis of inorganic pyrophosphatases begins just after fertilization in both categories of embryos. Noteworthy is the fact that in other enzymatic systems, the haploid embryos show differences in the emergence of some enzymes and enzymatic molecular forms during certain embryonic stages, as against the diploid embryos [13], [15].

The fluctuations of the enzymatic activity during different stages of embryonic development, which are similar for haploid and diploid embryos, are the result of the different metabolic rates of various metabolic pathways and of certain dissimilar regulating mechanisms accompanying the morphogenetic and the cellular differentiation processes.

REFERENCES

1. Beloff R. H., 1959, J. exp. Zool., **140**, 493.
2. Bucke C., 1970, Phytochem,., *9*, 1303.
3. Fankhauserg G., 1945, Quart. Rev. Biol., **20**, 20.
4. Fiske C. H., Subbarow Y., 1925, J. biol. Chem., **66**, 375.
5. Fox H., Hamilton L., 1964, Experientia, **20**, 289.
6. Gallien L., 1967, J. Embryol. exp. Morphol., **18**, 401.
7. Hamilton H., 1963, J, Embryol. exp. Morphol., **11**, 267.
8. Irie M., Iobuta A., Negi T., Tomito K., 1970, J. Biochem., **67**, 59.
9. Kornberg A., 1962, *Horizons in Biochemistry*, Acad. Press., New York.
10. Meșter R., Niculescu S., Scripcariu D., 1973, Rev. roum. Biol. Série Zool., **18**, 217.
11. Naganna B., 1950, J. biol. Chem., **183**, 693.
12. Naganna B., Isipati C. E., 1954, Nature, **174**, 593.
13. Preda V., 1969, *Biochimia dezvoltării embrionare la vertebrate*, Ed. Acad., București.
14. Rip J. W., Rauser W. E., 1962, Phytochem., **11**, 635.
15. Scripcariu D., Meșter R., 1973, St. Cerc. Biol. Seria Zool. , **25**, 223.
16. Soodsma P. I., Nordlie R. C., 1970, Biochim. biophys. Acta, **122**, 510.

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HISTOCHEMICAL RELATION BETWEEN DIHYDROFOLATE REDUCTASE AND FOLIC ACID IN THE RAT GASTRIC MUCOSA

BY

DOINA ONICESCU, IOANA CUIDA and MARIA POPESCU

By means of two original histochemical methods, the rat gastric mucosa was investigated as far as the folate metabolism was concerned.
The most characteristic are the proper gastric glands, showing a great concentration of folic acid and dihydrofolate reductase in the parietal cells. The pyloric glands appear with different degrees of intensity of folate and folate enzyme from one cell to another within the same gland.

Rat gastric mucosa was investigated concerning morphology [7], histoenzymology [4] [5] [12] and especially experimental physiology [9] [10] or pathology, often in connection with the gastric ulceration [1]. The present study describes the light microscopic features of some aspects of folate metabolism in the rat gastric mucosa.

MATERIAL AND METHOD

Male adult rats weighing from 200 to 250 g were used. Fragments collected from the corpus and the pyloric area of the stomach were cut in the cryotome (−25°C) and the 3 to 6 microns sections were introduced into the medium for dihydrofolate dehydrogenase [11] and in an original medium for folic acid, according to the following operations :

1. Incubation of fresh or deparaffinated sections in 0.004% potassium permanganate for 5 to 10 minutes.
2. Optional quick washing in distilled water.
3. Incubation of sections in a reagent prepared by mixing equal volumes of 0.002% sodium nitrite with 5 N HCl for 5 to 10 minutes.
4. Immersion of the sections in 0.05% sulphamic acid until the evolution of NO_2 bubbles ceases.
5. Immersion of the sections for 15 min at 37°C in a 0.001% solution of N-(1-naphtyl)-ethylendiamine dihydrochloride followed by development of purple azoderivative.
6. Immersion in Fast Blue B for reinforcing the brown reactive granules.
7. Quick washing in distilled water.
8. Three to five min immersion into 10% formalin.
9. Mounting of sections in glycerin of Apathy syrup. The reaction appears as purple-brown granules.

RESULTS

DIHYDROFOLATE REDUCTASE

Corpus of the stomach. The surface epithelium displays an enzyme activity of mean intensity. In lamina propria, the gastric glands show a very intense reaction in the parietal cells, but of weak positive one in the

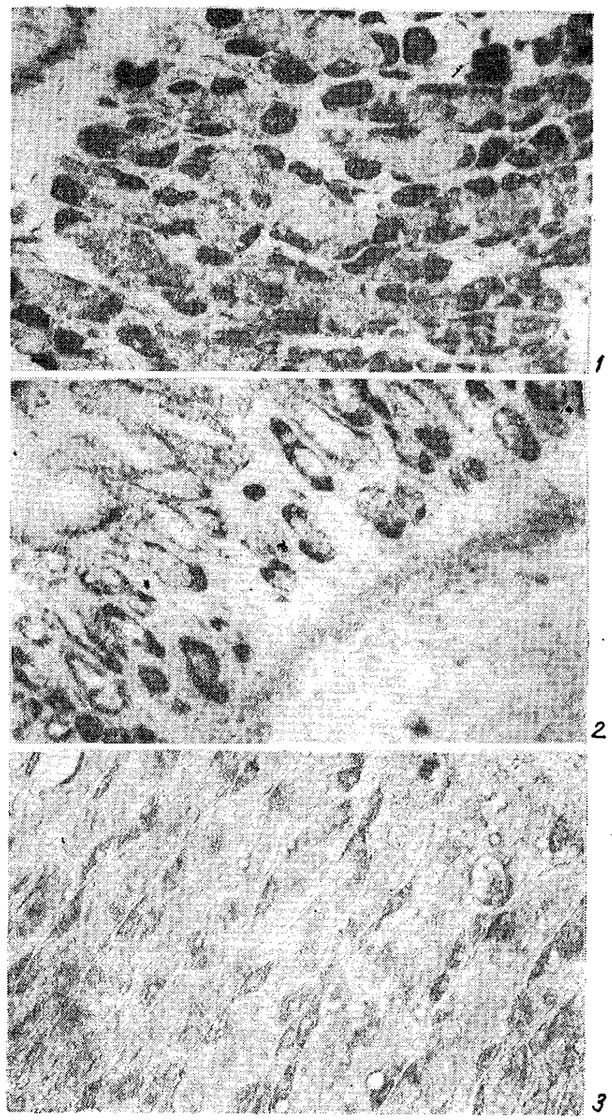

Fig. 1. — Dihydrofolate reductase. The gastric glands appear with a very intense reaction in the parietal cells and a weak activity in the zymogenic cells.

Fig. 2. — Dihydrofolate reductase. The pyloric glands show, in the same gland, different degrees of folate enzyme activity, from one cell to another.

Fig. 3. — Folic acid. An intense granular reaction appears in the parietal cells and a mean reaction in the chief cells.

zymogenic cells (Fig. 1). The muscle fibers of muscularis mucosae exhibit a mean enzyme activity.

The pyloric area. The epithelial cells of the mucosa are present with an intense enzyme activity. The gland cells show different degrees of folate enzyme in the same gland from one cell to another (Fig. 2). Muscularis mucosae shows a weak activity in the myocytes.

THE FOLIC ACID

Corpus of the stomach. The granular reaction appears especially in the parietal cells of the proper gastric glands (Fig. 3). The others structures of the mucosa display a weak reaction for the folates.

The pyloric area. The folic acid appears localized in some glandular cells of the pyloric region. In the same gland, areactive cells can be seen, near the very intense cells. The surface epithelium and muscularis mucosae display a weak reaction.

DISCUSSIONS AND CONCLUSIONS

The folate metabolism is obvious in the rat stomach mucosa, especially in the fundic and pyloric glands. The surface epithelium and the muscle fibers of muscularis mucosae have a weak folate metabolism. In the proper gastric glands, the parietal cells are the principal site of folate concentration and of the principal folate enzyme activity (dihydrofolate dehydrogenase).

But at the same time, in the majority of mammalian species (rabbit, monkey, ox, human), the parietal cells are the place wherefrom the intrinsic factor originates [2] [3]. According to Hoedemaeker et al [6], in the rat and the mouse, the chief cells seem to be the principal site of Castle's factor synthesis.

Experiments carried out in rats showed that, besides the synthesis of the intrinsic factor, the fundus is also required for normal B_{12} absorption [13], radioautograph confirming the localization of this vitamin in the fundic region.

Based on these last affirmations, on our own findings and on the fact vitamin B_{12} is associated with the folic acid in the biological processes, we suggest that even as far as the rat is concerned one can take into consideration the possibility the synthesis of the intrinsic factor could be carried out within the parietal cells and/or within the zymogenic ones, this process appearing sometimes more accentuated in the parietal cells. The molecules produces this way could be nonidentical [6].

Contrarily to Hoedemaeker et al [6], who sustain that, with each species, there is only one cell type which produces the Castle factor, we suggest three possibilities to be considered, according to which the intrinsic factor would originate in : 1. *the parietal cells* (human, guinea pig, rabbit, cat, monkey, ox) ; 2. *the chief cells* (mouse, rat) ; 3. *both cell types* (hog, rat).

The pyloric glands are characterized by different levels and degrees, respectively, of dihydrofolate reductase and folate reaction in the gland cells, thus suggesting the existence at the level of each gland of a differentiated functional cell cycle.

REFERENCES

1. Ader R., 1971, *Experimentally Induced Gastric Lesions* in : *Duodenal Ulcer*, ed. by H. Weiner, 1—39.
2. Boass A., Wilson T. H., 1964, Amer. J. Physiol., **206**, 783—786.
3. Deren J. J., 1973, Amer. J. Physiol., **225**, 1351—1354.
4. Etzler M. E., Birkenmeier H. E., Moog F., 1969, Histochemie, **20**, 99—104.
5. Hakanson A. D., 1967, Experientia, **24**, 666—667.
6. Hoedemaeker P. J., Abels J., Wachters J., Arends A., Nieweg H. O., 1966, Lab. Invest., **15**, 1163—1173.
7. Ito S., 1961, J. Cell. Biol., **11**, 333—347.
8. Johnson L. R., Grossman M. I., 1971, Gastroenterology, **60**, 120—144.
9. Konturek S. J., 1968, Scand. J. Gastroenterology, **3**, 572—576.
10. Martin F., McLeod J. B., Sircus W., 1970, Gastroenterology, **59**, 437—444.
11. Onicescu D., Popescu M., Tașcă L., Stoica I., 1972, Histochem. J. (London), **4**, 87—90.
12. Sung G. P., Jenkins B. C., Racey Burns L., 1973, Amer. J. Physiol., **225**, 1359—1363.
13. Wiseman G., 1964, *Absorption from the Intestine*. Academic Press, London—New York.

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ZONE CRITIQUE DE LA TEMPÉRATURE ATMOSPHÉRIQUE POUR L'ADAPTATION THYROÏDIENNE CHEZ LES BOVINS

PAR

GRAZIELLA NICOLAU et V. TEODORU

In cows of the Brown race, maintained in natural conditions with strictly constant diet, were studied during the cold season (November-April) the variations of the thyroid function (the level of the Protein-bound-iodine and the test of erithrocyte uptake of ^{131}I labelled T_3) in relation with the low amplitude spontaneous oscillations of the environmental temperature. The variations of the both mentioned thyroid indices show that the adaptation reactions of the thyroid are not equal in the different zones of the temperature scale; a "critical zone" appears near 0°C, in which the thyroid response — inversely related to temperature oscillations — is particularly prompt and statistically highly significant.

La stimulation de la fonction thyroïdienne par l'exposition au froid des organismes mammifères — réaction qui s'ensuit à l'utilisation intensifiée des hormones thyroïdiennes dans les tissus — a été mise en évidence dans de nombreuses recherches en utilisant différentes méthodes expérimentales; l'influence des températures élevées paraît avoir un sens opposé et provoquer, dans la plupart des cas, une baisse de l'activité thyroïdienne [10] [15]. Nous considérons cependant que, au point de vue biométéorologique, un fait est à souligner : les recherches concernant l'action de la température sur la thyroïde ont utilisé, dans la majorité des cas, des conditions artificielles — l'application aiguë du froid ou de la chaleur. En ce qui concerne la biométéorologie zootechnique, des études faites sur les bovins ont été concentrées essentiellement sur les tolérances et l'adaptation à des limites thermiques élevées [2] [5] [11] [12].

En utilisant des bovins adaptés à des conditions géo-climatiques différentes de celles qui existent en Roumanie, Johnson [4] a trouvé que pour l'organisme de vache « la zone de confort thermique » est située entre 0°C et +21°C.

Nous avons essayé d'établir le caractère du biorythme saisonnier et des variations spontanées de la fonction thyroïdienne, dans des conditions naturelles avec des variations thermiques d'amplitudes réduites, chez des animaux de laboratoire, bovins et ovins [6—9] [13]. En continuant ces recherches, nous nous proposons d'approfondir le problème du rapport entre les variations spontanées de la température atmosphérique et l'activité thyroïdienne des bovins. Dans ce but nous avons choisi l'intervalle thermique de −5°C à +15°C qui d'après nos recherches antérieures a été—au moins pour la race Brune, acclimatisée aux conditions de notre pays — le plus intéressant par rapport à la réponse de la thyroïde.

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MATÉRIEL ET MÉTHODE

On a effectué les recherches sur un groupe de 5 vaches de race Brune, d'environ 3—4 ans, en dehors des périodes de gestation et d'allaitement. Un mois avant le début des investigations, et pendant toute leur durée (de novembre jusqu'au mois d'avril) les animaux ont été logés dans la même étable, sans chauffage et aux fenêtres constamment ouvertes. Pendant ce temps la nourriture des animaux fut strictement standardisée.

Pour apprécier la fonction thyroïdienne on a utilisé le dosage d'iode protéique dans le sang (protein bound iodine — PBI) et l'épreuve Hamolsky, où l'on mesure la captation in vitro de la tri-iodothyronine marquée de l'^{131}I (T_3—^{131}I) par les hématies. Pour doser l'iode protéique dans le sang on a employé la méthode de l'incinération alcaline [14]. La détermination de la captation in vitro de la T_3—^{131}I par les hématies a été exécutée d'après la technique Hamolsky [3], adaptée par Berlescu et collab. (1).

Les prélèvements de sang pour les deux épreuves ont été faits 14 fois à différents intervalles de temps, pendant la période novembre—avril, sur tous les 5 animaux le même jour et chaque fois à 9 heures du matin. On a obtenu ainsi 70 déterminations pour chacun des deux indices thyroïdiens. Pour confronter les données biologiques et thermiques on a considéré les températures moyennes des 24 heures antérieures aux moments du prélèvement du sang. Les valeurs obtenues pour les deux indices thyroïdiens déterminés ont été groupés par rapport aux 4 zones de température, délimitées conventionnellement. De cette façon on a obtenu les séries d'investigations suivantes: 19 investigations correspondant à des températures atmosphériques de —5°C à 0°C, 19 pour les températures de 0°C à +5°C, 16 — de +5°C à + 10°C, et 16— de + 10°C à +15°C.

RÉSULTATS

La distribution des valeurs de l'iode protéique est représentée dans la figure 1. Les moyennes de ces valeurs (µg/100 ml) dans les 4 zones de température sont les suivantes: $6,9 \pm 0,27$; $5,1 \pm 1,01$; $4,6 \pm 0,47$; $3,9 \pm 0,49$.

On se rend compte aisément de la différence nette, significative d'ailleurs du point de vue statistique ($p<0,02$), entre les valeurs de l'iodémie qui correspondent aux températures négatives et les valeurs de l'iodémie de la zone toute proche des températures de 0°C à + 5°C. Les valeurs maximales, donc l'intensification nette de la fonction thyroïdienne, correspondent aux températures les plus basses. Dès que les températures dépassent +5°C, la tendance de diminution de l'iodémie, bien qu'existant encore, devient très discrète et ne se montre plus « couverte » statistiquement ($p> 0,05$).

La figure 2, représentant la distribution des valeurs du test Hamolsky, a beaucoup de ressemblances avec la première. La diffférence nette ($p < 0,003$) entre les valeurs des deux premières séries indique une réaction accentuée et constante de la fonction thyroïdienne par rapport à la transition des températures atmosphériques des valeurs négatives aux valeurs dépassant 0°C. La diminution de la captation de la T_3—^{131}I reflète la diminution de l'activité thyroïdienne pendant cette transition. Lorsque les températures dépassent +5°C, le test Hamolsky ne subit plus de modifications significatives du point de vue statistique ($p > 0,05$). Les moyennes des valeurs de la captation de la T_3—^{131}I (%) dans les 4 zones de température ont été les suivantes: $10,8 \pm 0,20$; $8,5 \pm 0,28$; $8,2 \pm 0,34$; $8,0 \pm 0,34$.

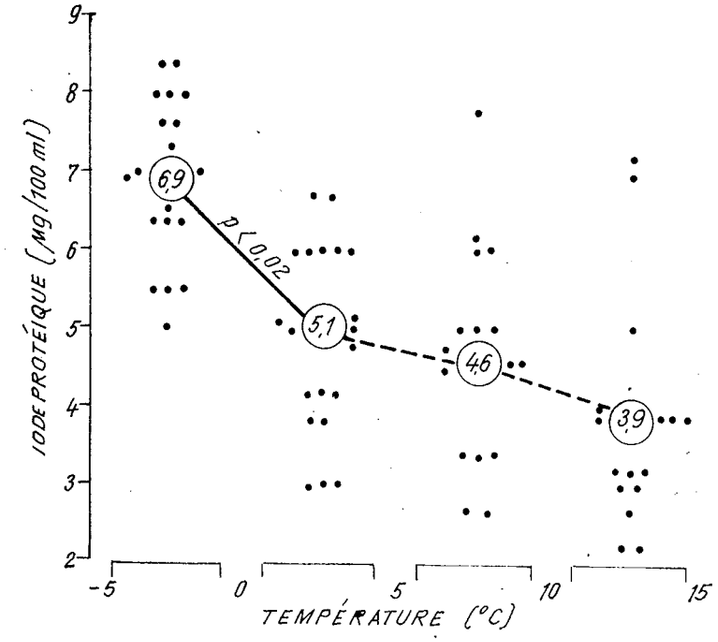

Fig. 1. — Distribution des valeurs de l'iode protéique par rapport aux 4 zones de la gamme des températures enregistrées pendant les investigations.

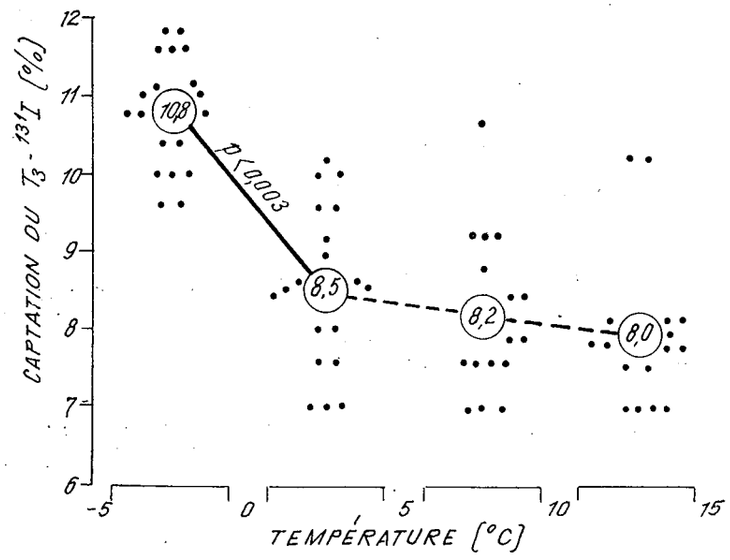

Fig. 2. — Distribution des valeurs de la captation *in vitro* de la T_3—^{131}I par les hématies, par rapport aux 4 zones de la gamme des températures enregistrées pendant les investigations.

CONCLUSIONS

Les résultats obtenus nous permettent de tirer les conclusions suivantes :

— La concordance des résultats des deux tests appliqués met en évidence l'existence d'une réactivité thyroïdienne par rapport aux oscillations naturelles de la température atmosphérique.

— Les réactions d'adaptation de la thyroïde ne sont pas égales et uniformément proportionnelles dans différentes zones de variation de la témperature atmosphérique.

— La prompte adaptation de la fonction thyroïdienne aux variations de la température ambiante se manifeste très nettement de $-5°C$ à $+5°C$; quand la température dépasse $+5°C$, il n'y a plus une corrélation statistiquement valable. L'influence sur la thyroïde pourrait être mise en évidence dans le cadre de certaines oscillations plus amples de la température — les oscillations saisonnières.

— Nos résultats nous suggèrent que, lorsque la fonction thyroïdienne s'adapte par rapport à la température ambiante, il existe une zone critique, située pour les bovins autour de 0°C, dans laquelle la thyroïde réagit d'une façon particulièrement rapide et énergique.

BIBLIOGRAPHIE

1. Berlescu E., Bădescu M., Georgescu Gh., Toma E., St. cerc. endocr., 1964, **15**, *4*, 333.
2. Bonadonna T., 1967, Zootecn. e veterin., 1967, **22**, *3—4*, 52.
3. Hamolski M. W., Golodetz A., Freedberg S., 1959, J. Clin. Endocr. Metab., 1959, **19**, *1*, 103.
4. Johnson H. D., 1965, Intern. J. Biometeorology, 1965, **9**, *2*, 103.
5. Lee D., 1965, Intern. J. Biometeorology, 1965, **9**, *1*, 29.
6. Lungu Al., Nicolau G., Cocu Fl., Teodoru V., Dinu I., 1966, Rev. Roum. Endocrinol., 1966, **3**, *4*, 279.
7. Lungu Al., Nicolau G., 1974, Rev. Roum. Endocrinol., 1974, **10**, *4*, 365.
8. Lungu Al., Nicolau G., Sucmanschi M., Teodoru V., 1967, Comunic. Conf. Fiziol., București, 1967, 107.
9. Lungu Al., Nicolau G., Teodoru V., 1969, Biometeorology, 1969, **4**, 5.
10. Macfarlane W. V., 1963, Rev. Res. UNESCO, Paris, 1963, 153.
11. Mahadevan P., 1968, in *Agroclimatological methods*, UNESCO, Imp. Réunies Chambery, 1968, 115—123.
12. McDowell R. E., 1966, World Rev. Anim. Prod., 1966, *1*, 39.
13. Nicolau G., Teodoru V., 1975, Rev. Roum. Biol.—Biol. Anim., 1975, **20**, *2*, 141.
14. Stoló V., 1966, *Estimation of iodocompounds in biological material*, Publishing House of the Slovak Acad. of Sci., Bratislava, 1966.
15. Tromp S. W., 1963, *Medical Biometeorology*, Elsevier, Amsterdam, 1963.

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CHROMOSOME STUDIES IN THE DIGENETIC TREMATODE *CEYLONOCOTYLE DICRANOCOELIUM* (FISCHOEDER 1901) NASMARK 1937

BY

P. VENKAT REDDY and S. SUBRAMANYAM

The digenetic trematodes constituting the largest group of Platyhelminthes (over 40,000 species) could serve as potential experimental animals; however, they have not been thoroughly investigated for their cytology, so far. While in some forms few details are available, in others lot of ambiguities exist about the chromosome numbers, their morphology and classification. Further there is a lacuna in our knowledge about these aspects in other unexplored parasites. Some attempts have been made recently in this direction with *Philophthalmus* sp. [14], *Fasciola gigantica* [15] and *Paramphistomum cervi* [16].

The Paramphistomes are economically important from medical as well as from veterinary points of view because of their ubiquitous infestation of animals and humans. There is no reliable description of the chromosome behaviour and the karyotypes of almost all the species belonging to the family Paramphistomatidae [2] [3] [7—11] [13] [18—20].

The genus *Ceylonocotyle* [4] [6], consisting of five species, has not been studied in detail except for the brief report of Sharma et al [7] which mentions only the chromosome numbers of *C. orthocoelium*, *C. dawesi* and *C. scoliocoelium* as 14, 20 and 22, respectively. The diploid chromosome number of *C. dicranocoelium* has been established for the first time to be eighteen.

MATERIAL AND METHODS

The adult parasites of *C. dicranocoelium* collected from the rumen of cattle was processed by the technique described by us, elsewhere [14] [15], with the modification that the divisional stages were scored from aceto orcein squashes of the tested material instead of the haematoxylin preparations. The constructions of the karyotype has been made from gonial metaphases. The accuracy of the diploid chromosome number has been confirmed from a count of the meiotic bivalents. Chromosome measurements, their relative lengths, arm ratios and the centromeric index have all been made from photomicrographs. The construction of the idiogram and the terminology used were essentially the same as described by Levan et al [5].

RESULTS

Large number of metaphase plates have been screened to establish the diploid chromosome number of C. *dicranocoelium* as eighteen. Six gonial metaphases have been used for measurements for the karyotypic

analyses. The average data of these latter are presented in table 1 using the three morphological criteria as enunciated by Volpe and Bebhardt [17].

Table 1

Chromosome measurements of *C. Dicranocoelium*

Chromosome number	1	2	3	4	5	6	7	8	9
Relative length *	17.0	14.8	13.5	11.1	10.8	10.2	8.7	7.6	6.8
Arm ratio**	6.7	1.6	3.9	2.9	1.0	2.1	1.8	1.9	1.8
Centromere index ***	13.3	39.4	21.5	30.5	49.9	33.1	36.3	34.3	37.1

* Length of chromosome / total length of haploid genome × 100
** Length of long arm / length of short arm
*** Length of short arm × 100 / length of whole chromosome

The idiogram constructed from the above data is shown in figure 2. The chromosomes are mostly submetacentric except two acrocentric pairs and one metacentric pair. They could be arranged into three groups in the descending orders of their lengths.

Group I has three pairs of relatively long chromosomes of which pairs 1 and 3 are acrocentric and pair 2 is submetacentric.

Group II possesses three pairs of medium sized chromosomes of which the fourth and the fifth pair in the series may almost approximate each other in their lengths. However they could be readily distinguished from the centromeric position in that the fifth pair is metacentric. The sixth pair is again submetacentric.

Group III comprises three pairs of small submetacentric chromosomes. The ratio between the longest and the shortest chromosome is 7.7/3.1 = = 2.48 μ. Although early meiotic stages could not be clearly delineated, late diakinesis stages where the bivalents were distinctly seen helped in confirming the chromosome number (Fig. 3).

DISCUSSION

The literature available includes the brief mention of Sharma et al [7] indicating the chromosome numbers of *C. orthocoelium*, *C. dawesi* and *C. scoliocoelium* as 14, 20 and 22, respectively. Among the other two species left unexplored, the chromosome number of *C. dicranocoelium* has been reported here as 2n=18 and confirmed from the meiotic studies. The karyotype and the idiograms have also been provided (Figs. 1—3). Thus variations seem to be present within the same genus. Although Short and Menzel [12] are of the opinion that only generic boundaries could be established but not at the species level, the cytological data available at the moment leaves one to presume that the species of *Coelonocotyle* could be recognized merely on the basis of the chromosome numbers. The chromosome variations seen in this genus may be due to the gradual addition of chromosomes. It is, therefore, suggestive that aneu-

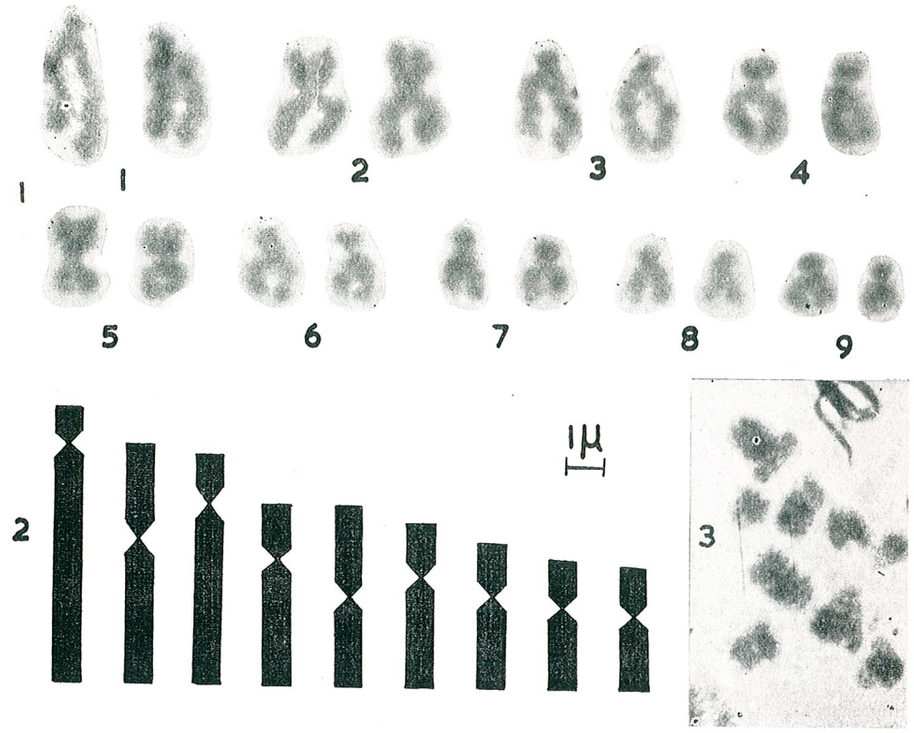

Fig. 1—3. — *1*, Karyotype of *C. dicranocoelium*. *2*, Idiogram. *3*, Late diakinesis showing the nine bivalents. × ca. 2,300.

ploidy may operate as a major evolutionary factor in the digenetic trematodes as originally postulated by Britt [1].

Studies on the karyotypic variations which could possibly establish subtle differences between genera and species have not been given any importance. Such investigations in any genus or family could be assessed only when the karyotypes of most of the forms are readily available. The application of comparative cytology to systematic relationships may perhaps be helpful to study the evolutionary patterns in the family Paramphistomatidae and other digenetic trematodes rather than merely basing the differences on the morphological features.

CONCLUSIONS

The chromosome number of the digenetic trematode *Ceylonocotyle dicranocoelium* has been established from the aceto orcein squashes of gonial metaphases to be 18 as the diploid number. This is confirmed from the meiotic stages also. They consist of three pairs each of large, medium and small sized chromosomes. Except the first and the third pairs which are acrocentrics and the fifth metacentric pair belonging to the medium sized category the rest are all submetacentrics. The karyotype and the idiogram are presented.

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REFERENCES

1. Britt H. G., Amer. Nat., 1947, **81**, 276.
2. Dhingra O. P., Res. Bull. Punj. Univ., 1955a, **64**, 1.
3. — Res. Bull. Punj. Univ. 1955b, **65**, 11.
4. Gupta N. K., Res. Bull (N.S.), Punj. Univ., 1965, **16**, 277.
5. Levan A., Karl F., Sandberg A. A., Hereditas., 1964, **52**, 201.
6. Nasmark K. E., Zoologiaka Bidr. Uppsala, 1937.
7. Sharma G. P., Mittal O. P., Madhu Bala, Proc. 55th Ind. Sci. Cong. Part III, Absts., 1968, 472.
8. Srivastava M. D. L., Jha A. G., Natl. Acad. Sci. Ind. Annual No., 1964, 126.
9. Saksena J. N., Proc. Nat. Acad. Sci., India, Sect. B., 1962, **32**, 177.
10. — Proc. Nat. Acad. Sci., India, Sect. B., 1969, **39**, 81.

11. Sey O. Acta. Vet. Hung., 1971, **21**, 93.
12. Short R. B., Menzel M. Y., Jour. Parasitol., 1960, **46**, 273.
13. Van der Woude, Amer. Midland Naturalist., 1954, **51**, 172.
14. Venkat Reddy P., Subramanyam S., Current Sci., 1971, **40**, 578.
15. — Current Sci., 1973, **42**, 288.
16. — 1974 (in press).
17. Volpe E. P., Bebhardt B. M., Copeia, 1968, *3*, 570.
18. Willey C. H., Godman G. C., Jour. Parasitol., 1951, **37**, 283.
19. Willmott S., Jour Helminthol., 1950a, **24**, 1.
20. — Jour. Helminthol., 1950b, **24**.

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CYTOGENETIC STUDIES IN BOVINE LEUKOSIS

BY

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Investigation bearing on some blood constants (proteinemia, hemoglobinemia, hematocrit, albuminemia, globulinemia) carried out in cows with leukosis and in normal control animals showed no significant differences between these two groups. Examination of the cytogenetic changes occurred in bovine leukosis revealed a reduction of the normal euploid cellular line to 64.5% by an increase of aneuploidy. There was also an inconstant numerical variation of chromosomes. In the cellular line having 2n = 59 (which represented 7.2% of the whole cell population), in 38% of the metaphases a new submetacentric chromosome, in addition to sex chromosomes, was observed. The features of chromosomal changes in bovine leukosis due to the lack of constancy and specificity remind the human lymphosarcomas and also the tumoral cytogenetic evolutions.

Researches carried out in bovine leukosis [1] [3] [5] [7—10] describe the following chromosomal features: inconstant number variations, irregular presence of an autosomal submetacentric chromosome, stem lines with different chromosomal complements as compared to the euploid one, etc.

In this report, the cellular changes observed in bovine leukosis are presented.

MATERIAL AND METHOD

The studies were achieved in 15 cows with lymphocytes leukosis clinically and hematologically established and in 15 healthy control animals. The leukocyte formula and some hematological indices, such as: total proteinemia, hemoglobin content (g/100 ml), hematocrit, average erytrocytes hemoglobin concentration, albumin and globulin content (g/100 ml) were studied.

The total plasma proteinemia was refractometrically determined by using the refraction indices and the corresponding values from the tables [11]. The hemoglobin assay was carried out by the photometric method with cyanmethemoglobin and the hematocrit by Wintrobe's macromethod.

The hemoglobin and hematocrit values used to calculate the average erytrocytes hemoglobin concentration.

Paper electrophoresis was employed to separate the serum protein fractions. After elution in NaOH 0.1N, the electrophoregrams were interpreted at Lange's photocolorimeter. Based on the colorimetric indices fractions values were calculated in g/100 ml of serum.

In order to observe the cytogenetic changes occurring in leukosis, 970 mitoses coming from leukotic cows and 1,036 mitoses coming from controls were cytogenetically examined. The distribution into frequency classes of dividing cells, according to the chromosome number, as well as the structural changes occurred, were followed up.

For obtaining chromosomal smears, the short-term whole blood culture method was used. It consisted of 10 ml IC 65 culture medium *, 0.8 ml of normal calf serum, 0.2 ml phyto-

* The tissue culture medium was prepared by the "I. Cantacuzino" Medical Research Institute, Bucharest—Romania.

hemagglutinin M and 0.1 ml phytohemagglutinin P for 1 ml of whole blood. The slides have been examined and photographed by means of the research microscope MC_1 on DK_3 film and BC_1 paper.

RESULTS AND DISCUSSIONS

The hematologic diagnosis of leukosis carried out according to the Bendixen key and confirmed subsequently from the clinical and histological viewpoint, revealed a pronounced increase of the white series varying between 14,000 and 19,000 (normal limits 7,000—9,000 for the *Bos taurus L* species).

The leukocyte formulas have shown a clear lymphocytosis with variations between 70% and 90% as compared to 60% found in normal animals. The values of the hematologic indices are presented in table 1.

Table 1

Hematological indices in control and leukotic animals

	Hemoglobin (g/100 ml of blood)	Hematocrit	Average erytrocyte hemoglobin (CHEM)	Proteinemia (g/100 ml of blood)	Albumin (g/100ml)	Globulin		
						α	β	γ
LEUKOSIS	12.73	34.53	36.59	8.84	3.51	1.14	1.03	3.15
CONTROL	11.71	32.13	36.24	8.52	3.27	1.32	1.04	2.88

Total plasma proteinemia in leukotic individuals presented a larger value than in controls. This fact might be explained by a more intense synthesis activity which characterizes a proliferative process. Nevertheless these values have been found within the normal limits of this species.

The hemoglobin concentration (g/100 ml) in leukotic individuals was slightly increased, thus probably compensating the numeric anemia which usually accompanies leukosis. However the hemoglobin values remain within the normal limits for this species. The hematocrit, the average hemoglobin and the albumin and globulin concentration have shown a difference between the two groups but did not exceed the normal limits of the species. The assay of the differences between the values of these indices in the two groups by means of the Student's "t" test revealed the following : significant differences for hemoglobin, proteinemia, albumin and gamma globulin ($0.05 < P < 0.1$), highly significant differences for the hematocrit and the alpha globulin ($0.01 < P < 0.05$) and nonsignificant differences for average hemoglobin and the beta globulin content ($P > 0.1$).

The analysis of the cytogenetic changes occurred in the leukotic cells has shown a larger dispersion around the modal number (50 to 120 chromosomes) than in the cells of the control population (Fig. 1). Nevertheless, the euploid line (2n = 60) remains the stem line representing 64.5% of the leukotic cell population. In control animals the euploid line represented 89.5%. The numerical reduction of the euploid line of the leukotic cell population was due to the increase of aneuploidy. Three cell lines become predominant within this latter :

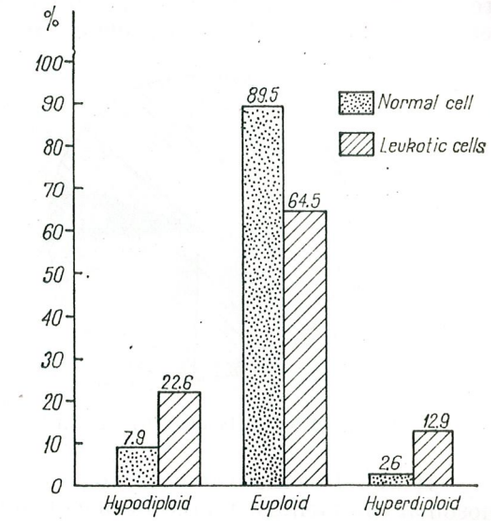

Fig. 1. — Cell distribution according to chromosome number in control and leukotic cells.

— the tetraploid or almost tetraploid cell line (7.3%) ;
— the cell line with 2n = 59 (7.2%) and
— the cell line with 2n = 61 (4.3%) (Fig. 2).

The two ratios equality test was used to compare the two cell populations (the normal and the leukotic ones). The differences were highly significant ($P < 0.001$).

In addition to this numerical disorder, the presence of a new big submetacentric chromosome, alongside of the two sex chromosomes, was observed in 38% of the cell line with 2n = 59 (Fig. 3).

Since the frequency of this new chromosome in the whole cell population is rather low (2.7%), no conclusion can be drawn for the moment. However, the occurrence of this possible "chromosome marker" in 7% of the cells of one of the animals studied leads to the presumption that the chromosomal configuration reflects certain metabolic disturbances which appear in a certain stage of the disease evolution.

The coexistence of more cell lines having different chromosome complements alongside with the euploid lines as well as the irregular occurrence of some new chromosomes (submetacentric, dicentric, etc.) somewhat reminding the observations carried out in human leukemias and especially in tumors, represent together the picture of changes which affect the chromosomal complement in bovine leukosis.

The chromosome changes, considered as epiphenomen, showing no relation with the development of leukosis do not agree with the observa-

tions made in human chronic granulocytic leukemia, where a constant specific chromosome abnormality was identified, namely the Ph¹ chromosome.

Neither the hypothesis according to which the chromosome changes are a cause of leukemogenesis is justified, since in leukosis, the chromosomal behaviour is very different, being sometimes susceptible to change.

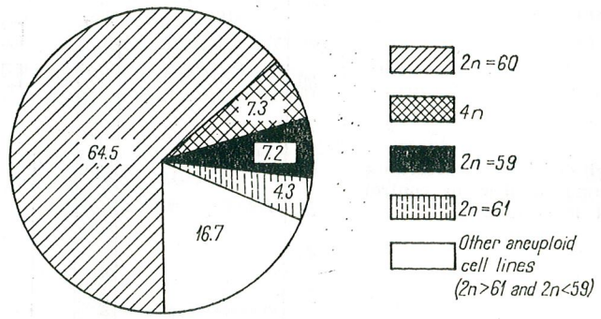

Fig. 2. — Ratio of different cell lines in leukotic cell population.

It would be more credible to assume that the anomalies of the chromosomal complement represent one of the factors favouring the occurrence and the development of leukosis.

A hypothesis was advanced in 1963 by Nichols [9] concerning a possible relationship between viruses, chromosomes and carcinogenesis. This hypothesis might also be extended to carcinogenesis induced by ionizing radiations and chemical substances, thus offering a general explanation to the way neoplastic characters are inherited through chromosomes.

REFERENCES

1. Basrur P. K., Gilman J. P. W., Mc Sherry B. J. 1964, Nature, **201**, 368—371.
2. Boveri T., 1902, Verhandl. Physik. Med. Ges. Würzburg, **35**, 67—90.
3. Gustavsson I., Rockborn G., 1964, Nature, **203**, 990.
4. Hamerton J. L., 1971, *Human cytogenetics*, Academic Press, New York and London, **2**, 407.
5. Hare W. C. D., Mc Feely R.A., Abt D. A., Feierman J. R., 1964, J. Nat. Cancer Inst., **33**, *1*, 105.
6. Makino S., 1957, Intern. Rev. Cytol., *6*, 25—84.
7. Marshak R. R., Hare W. C. D., Dutcher R. M., Schwartzman R. M., Switzen J. W., Hubben K., 1966, Cancer, **19**, 724.
8. Martin W. B., Flanagan M., 1967, Brit. J. Cancer, **21**, *1*, 137—143.
9. Nichols W. W., 1963, Hereditas, **50**, 53.
10. Weinhold E., 1970, *Chromosomuntersuchungen bei der Rinderleukose.*, Europ. Kolloquium Uber Zytogenetik, Giessen.
11. Weeth H. J., Speth C. F., 1968, J. Anim. Sci., **27**, *1*, 146—149.

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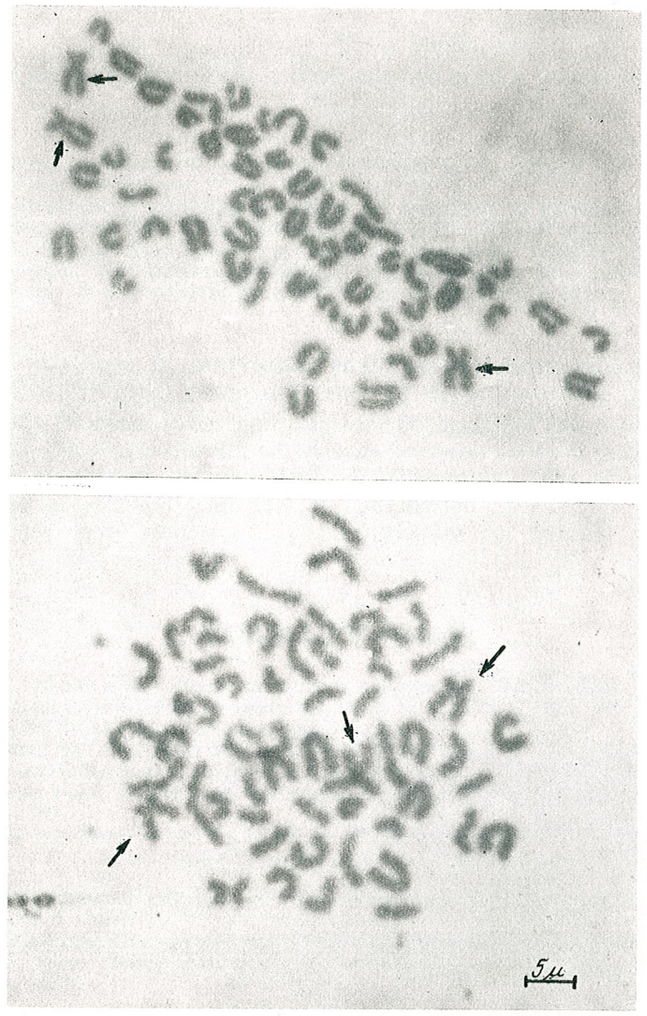

Fig. 3. — Dividing cell from cell line 2n = 59 with a new submetacentric chromosome.

GENETIC VARIABILITY OF HEMOGLOBINS AND SERUM TRANSFERRINS IN ŢURCANA SHEEP

BY

EUGENIA MILOVAN, I. GRANCIU and ELENA STAMATESCU

Starch gel electrophoresis was used to determine Hb in 285 and Tf in 268 ewes of Ţurcana breed from Runcu Experiment Station.
Nine Hb phenotype controlled by 4 alleles were identified. Hb B/B had the highest frequency (48 %) Hb F/F was also found in 9.5 % and Hb D/D in 1 % of the cases studied. Tf presented 17 phenotypes controlled by 8 alleles. Tf D/D (42 %) and his heterozygous combinations predominate over other types. Tf J and Tf L were found only in heterozygous state with a low (< 1 %) frequency.

The existence of individual variations in ewes concerning hemoglobin and serum protein was described by several authors [2], [3].

Electrophoretic investigations for the study of these variations also demonstrated the presence of differences between sheep breeds in gene and phenotype frequencies at the Hb and Tf level [1]. The objective of this paper is to present Hb and Tf types identified in Ţurcana breed and to establish the gene and genotype frequencies for further use in blood typing.

MATERIAL AND METHODS

Blood samples were collected on citrated anticoagulant solution from 285 ewes for the hemoglobin assay; for transferrins study the serum was collected from 268 ewes of Ţurcana breed belonging to the Runcu Experiment Station.

a) Starch gel electrophoresis based on a discontinuous buffer system was used [4]. The starch was of indigenous origin and was partially hydrolyzed in this laboratory.

For hemoglobin studies fresh erythrocytes were used. Lysis was done by dilution in distilled water 1/200.

The gel was prepared of 10 % starch in a buffer solution composed of Tris and citric acid at 8.5 pH. The electrolyte comprised 11.8 $g^0/_{00}$ boric acid at 8.2 pH corrected with lithium hydroxide.

Samples insertion was done at 7 cm distance from the catodic extremity of the gel. Migration took 4 hours.

b) For identification of the Tf types starch gel electrophoresis was used with 8.9 pH and 12% of starch. During seven hours of electrophoretic migration starch gel was cooled with running water.

RESULTS

a) *Hemoglobin types.* In the Ţurcana breed analysed 4 alleles (A, B, D and F) and 9 phenotypes of Hb were found facing three types usually described by literature (Fig. 1).

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The relative distribution of the genotypes observed is presented in table 1. No individuals possessing Hb A/D and Hb A/F were identified. However, in 4.56% of animals the presence of Hb D was noticed as previously described by Vaskov and Efremov [5] in Jugoslavian native ewes. We also found homozygous Hb D (7%) and Hb F with heterozygous combinations like Hb FB (12.28%) or Hb DF (0.7%).

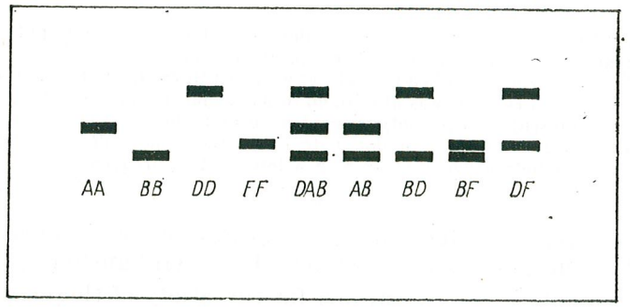

Fig. 1. — Diagram of hemoglobin types in Ţurcana breed.

The most frequent homozygous Hb genotypes in the Ţurcana breed were Hb B followed by Hb F and D. The lowest frequency was displayed by the Hb A type.

Table 1

The Hb phenotypes and their relative frequency in the Ţurcana breed

Phenotypes observed									
AA	BB	DD	FF	AB	DAB	BD	BF	DF	Total
1	137	2	27	38	13	30	35	2	285
0.35	48.07	0.70	9.47	13.30	4.56	10.52	12.28	0.70	99%

b) *Transferrin types* in the Ţurcana breed are represented by 8 alleles out of the 9 described by literature and by 17 identified genotypes (Table 2). Homozygous types were found for Tf A, B, C, D, E and M but no homozygous individuals for Tf J and L. Tf D had the highest gene frequency (42%) followed by the Tf B (25%). The most frequent genotypes were the heterozygous Tf B/D (21.5%) and the homozygous Tf D/D (18%). Very rare proved to be Tf J, L and M and their combinations.

Table 2

Allele frequency and genotypes repartition in the Ţurcana breed (n = 268)

Tf allele	Gene frequency	Genotype	Genotype frequency (%)
		AA	0.3270
A	0.180±0.0390	BB	0.0645
B	0.253±0.0373	CC	0.0060
C	0.078±0.0414	DD	0.1806
		EE	0.0016
D	0.425±0.0327	MM	0.0001
E	0.041±0.0422	AB	0.0919
		AC	0.0282
J	0.001±0.0431	AD	0.1538
L	0.007±0.0430	AJ	0.0007
		AM	0.0039
M	0.011±0.0429	BC	0.0396
		BD	0.2159
		BE	0.0208
		BL	0.0035
		CE	0.0063
		DE	0.0348

DISCUSSIONS AND CONCLUSIONS

Apart from the majority of sheep breeds which generally have two hemoglobin types (Hb A and B), in the Ţurcana breed four types and nine genotypes are identified.

The great number of hemoglobin types found in the Ţurcana breed might prove the existence of a great genetic variability. This situation makes it very useful to employ the hemoglobin types in blood typing and immunogenetic studies on the Ţurcana breed.

The presence of rare Hb F and Hb D types might be explained by the actual stage of improvement and by the robustnes which is proper to this native and well adapted breed.

To similar results also came the observations on serum transferrin in which the heterozygous types prevail (71%) as compared to the homozygous ones.

The great number of Tf and Hb genotypes in the Ţurcana breed suggests the existence of a high genetic heterogeneity and also leads to the conclusion that a systematic selection might have good chances to act efficiently for the improvement of the productive characters of this breed.

REFERENCES

1. Ashton G. C., Nature, 1963, **198**, 1117–1118.
2. Cabannes, R. Serain C., CR. Soc. Biol. 1955, **149**, 1193–1197.
3. Evans, J. V., King J. W. B., Cohen, B. L., Harris, H., and Waren, F. L., Nature, 1956, **178**, 849–850.
4. Smithies O.,Biochem. J., 1955, **61**, 629–641.
5. Vaskov B., Efremov, G., Nature, 1967, **216**, 513–594.

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LA RÉACTION TISSULAIRE SOUS L'INFLUENCE DE CERTAINS INSECTICIDES CHEZ *BOTHYNODERES PUNCTIVENTRIS* GERM.

PAR

MARIA TEODORESCU et VIORICA TRANDABURU

The nervous and genital tissues of *Bothynoderes punctiventris* undergoes heavy structural changes following assay with the Fosfotox and Heptaclor insecticides carried out in April and May — June periods.
In the nervous tissue, the maximal sensitivity is showed, in the first place, by glyocytes and tracheocytes, and secondarily by the associative neurons; the motoneurons appear altered only in the last phase and when submitted to lethal doses.
The desintegrated ovarian follicles are isolated and phagocytized by the follicular and vitellarium cells, a fact which has repercussions upon the insect's fecundity.
The experiences reveal the selective role of epithelia in the transfer of substances from the neurons and from ovocytes to the hemolymphe.

Certains chercheurs sont arrivés à la conclusion que les doses létales d'insecticides modifient directement la fécondité sans affecter le système nerveux [5]. Des données plus récentes signalent l'action du lindane, du parathion et du DDT, appliqués à *Periplaneta americana*, *Musca domestica*, *Mysus persicae* et *Acanthoscelides obtectus* [1] [2] [3] [4] [6] [7] [8] [9]. Le puissant effet des insecticides Heptaclor et Fosfotox R sur l'insecte *Bothynoderes punctiventris* ressort clairement si l'on poursuit la prolificité et l'indice de mortalité dans la période ovipositaire. *Bothynoderes punctiventris*, l'un des plus périlleux insectes nuisibles à la culture de la betterave à sucre, détermine la diminution de la production de cette dernière par hectare. La vérification histologique de la réaction de l'organisme de l'insecte aux insecticides fournit des précisions dans les directions suivantes : 1. L'établissement du degré de toxicité des différentes doses d'insecticides. 2. Le choix du moment le plus propice à l'application du traitement. Dans une note précédente, nous avons signalé des troubles de comportement chez *Bothynoderes* et la réaction de la gaine périneurielle à la sortie de la diapause et pendant la ponte, sous l'influence des insecticides Heptaclor et Fosfotox R [10].

Dans le présent travail nous continuons à poursuivre l'action des insecticides susmentionnés sur les systèmes nerveux et génital, en tâchant d'établir si l'éventuelle altération du système nerveux conditionne la modification morphophysiologique du système génital chez *Bothynoderes punctiventris*.

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MATÉRIEL ET TECHNIQUE

Nous avons appliqué sur l'articulation protororacicomésothoracique des insectes collectés pendant la période avril et mai — juin 2 microlitres de Heptaclor et Fosfotox R, à l'aide de la microseringue Agla. La dose sous-létale pour les deux insecticides a été de 0,025 microgrammes et la dose létale de 1,6 microgrammes pour le Heptaclor et de 8 microgrammes pour le Fosfotox R. Le système nerveux et le système génital ont été prélevés par microdissection, traités ensuite par les mêmes techniques histologiques courantes et étudiés sur des sections sériées.

RÉSULTATS

L'effet des insecticides sur la structure du tissu nerveux. Les dégénérescences observées sur ce tissu sont moins prononcées et moins fréquentes que dans le perineurium [10], ce qui conduit dès le début à la supposition que l'épithélium périneuriel, ayant le rôle de barrière sélective dans les échanges tissu nerveux-hémolymphe, enregistre le premier toutes les variations survenant dans la composition chimique de l'hémolymphe. Nous allons montrer plus loin que les gliocytes intraganglionnaires et les trachéocytes sont aussi sensibles que les épithéliums sélectifs.

Dans la zone corticale des ganglions nerveux des *Bothynoderes*, sous le perineurium, les neurones sont placés sur plusieurs rangs. Le neuropyle occupe la zone centrale et consiste en faisceaux de fibres nerveuses afférentes et efférentes. Dans la zone corticale et à la limite avec le neuropyle il y a de nombreux trachéoles de calibres variés. Les cellules gliales entourent les neurones, le neuropyle et les fibres nerveuses ; elles sont donc périneuronales, périneuropylaires et périaxonales.

Les expériences avec des doses sous-létales et létales de Heptaclor effectuées en avril. Les lésions affectent les neurones associatifs : la chromatine se disperse, les nucléoles s'agrandissent. Les pycnoses sont rares dans ces neurones, mais elles sont fréquentes en gliocytes et trachéocytes. En *mai* et *juin* la fréquence des noyaux pycnotiques s'accroît en neurones associatifs comparativement au mois d'avril. Les gliocytes et les trachéocytes sont également fortement altérés.

Les expériences avec la dose sous-létale et létale de Fosfotox effectuées en avril. Ces doses ont la même action violente sur le perineurium, les gliocytes intraganglionnaires et les trachéocytes. La dose sous-létale produit, dans une moindre mesure que la dose létale, la dégénérescence des neurones d'association et parfois celle des neurones moteurs. On rencontre des aspects d'agglutination partielle de la chromatine dans le centre du noyau et le stade final de pycnose complète. En *mai* et *juin*, les deux doses de Fosfotox déterminent une réaction plus intense du tissu nerveux des ganglions thoraciques et de la masse nerveuse abdominale et une réaction pycnotique presque généralisée chez les gliocytes et les trachéocytes.

Les aspects variés de dégénérescence des éléments du tissu nerveux ganglionnaire peuvent être résumés et interprétés de la manière suivante :

a. Dans toutes les expériences les gliocytes et les trachéocytes sont massivement altérés, c'est-à-dire justement les éléments qui assurent aux neurones les échanges trophiques et respiratoires. Ce sont les neurones associatifs qui dégénèrent notamment sous l'influence des doses létales de Heptaclor et des doses létales et sous-létales de Fosfotox.

b. Les motoneurones sont sensibles tout particulièrement à l'action de la dose létale; l'effet des doses sous-létales ne se produit que lorsque la motricité est affectée et la paralysie se déclenche.

c. Les effets constatés dans les cellules nerveuses peuvent être considérés comme secondaires étant donné que ce sont les éléments gliaux et les trachéocytes qui manifestent la première réaction aux insecticides. Le tissu nerveux maintient une réserve de cellules non altérées même dans des conditions précaires, ce qui lui confère une certaine résistance pendant toute la période consécutive au traitement ; ceci expliquerait aussi le retour à la normale de certains insectes traités par la dose sous-létale.

L'action des insecticides sur le système génital. A la sortie de l'insecte de la diapause, vers le bout distal du germarium se développent deux processus synchrones : l'ovogenèse et la différenciation des follicules ovariens. En reste, le germarium est occupé par des cellules à noyaux volumineux et peu de cytoplasme, séparées par des plages de matériel basophile. Les follicules du vitellarium ne peuvent pas pénétrer dans le pédicule de l'oviducte parce que le passage est bloqué par le bouchon folliculaire ; ce n'est qu'en mai que ce passage se produit normalement. L'épithélium folliculaire a des cellules hautes seulement après la diapause et très hypertrophiées et élargies pendant la ponte.

Les tests effectués en avril. Les doses sous-létale et létale de Heptaclor et celle sous-létale de Fosfotox déterminent, dans les follicules ovariens, la vacuolyse du nucléoplasme de l'ovocyte, la déformation du matériel nucléolaire, la prolifération de l'épithélium folliculaire et la transformation de certaines cellules phagocytaires en phagocytes, qui envahissent graduellement et phagocytent l'ovocyte (Pl. I, fig. 1—5). Le traitement au Fosfotox létal entraîne dans cette dégénérescence le germarium et l'épithélium du vitellarium ; en même temps le phénomène d'atrésie folliculaire s'accentue ; les épithéliums des follicules et du vitellarium s'unissent en une masse cellulaire qui obstrue la lumière du vitellarium ; de nombreuses cellules prolifèrent et, après avoir activé comme phagocytes, se fragmentent (Pl. II, fig. 6, 7).

Les tests effectués en mai — juin ont des effets dans toutes les expériences faites sur le germarium (Pl. II, fig. 8) et les follicules prévitellogènes ; les ovocytes en pleine vitellogenèse manifestent surtout la dégénérescence de quelques plaquettes vitellines. En dehors des phénomènes décrits ci-dessus apparaît aussi la vacuolyse ou la compression du cytoplasme des ovocytes (Pl. II, fig. 9). De même qu'au mois de mai, mais d'une manière beaucoup plus accentuée et plus fréquente, après le traitement au Fosfotox létal s'installe la dégradation des follicules ; les follicules amassés anormalement dans le vitellarium dégénèrent, le noyau des ovocytes entre en pycnose et les phagocytes folliculaires pénètrent dans le cytoplasme des ovocytes en voie de destruction (Pl. II, fig. 10, 11).

DISCUSSIONS ET CONCLUSIONS

Au cours de notre étude concernant les effets du traitement au Heptaclor et au Fosfotox sur le tissu nerveux et génital de *Bothynoderes punctiventris* pendant les mois d'avril et mai—juin, nous avons remarqué tout particulièrement l'évolution et la réaction de l'épithélium folliculaire par comparaison à l'épithélium périneuriel, que nous avons décrit dans un travail antérieur [10]. Les deux épithéliums ont le rôle de barrière sélective, contrôlant le passage de substances de l'hémolymphe dans les cellules limitrophes ainsi que dans le perineurium. Dans tous les cas expérimentaux nous avons constaté la réduction de la hauteur des cellules folliculaires, leur entrée en pycnose et la prolifération anarchique. Nous avons remarqué le dernier phénomène aussi dans le perineurium à une autre occasion, non pas comme réaction aux insecticides mais après la greffe de ganglions nerveux provenant d'autres insectes (travail non publié). L'intoxication avec des insecticides a déterminé également la prolifération de l'épithélium du vitellarium. Le dérèglement de l'équilibre dans les relations cellules folliculaires — ovocyte, cellules gliales — neurones, pourrait être corrélé aux effects des insecticides sur la prolificité [4]. Il ressort que les épithéliums périneuriel, folliculaire et du vitellarium peuvent servir comme indice morphologique sensible pour apprécier le degré d'intoxication de l'insecte. Ces épithéliums, les gliocytes et les trachéocytes subissent les premiers l'influence toxique, s'altèrent et, comme suite, les rapports fonctionnels spécifiques avec les cellules limitrophes se dérèglent, en l'espèce avec les neurones et les ovocytes. Envisagée de ce point de vue, l'influence des insecticides sur les cellules nerveuses et germinales nous paraît secondaire. On peut conclure que les insecticides étudiés ont généré non seulement des troubles de comportement [10] mais aussi des altérations graves au niveau morphofonctionnel dans le tissu nerveux et germinatif de *Bothynoderes*.

Les graves dégénérescences intervenues dans le tissu nerveux ont des répercussions directes sur le développement de la folliculogenèse et de la prolificité de l'insecte.

Fig. 1. — Heptaclor sous-létal, avril. Follicule prévitellogène ; phase de début de la dégénérescence du noyau. Zone périnucléolaire condensée (*c*) et zone périphérique laxe (*l*).
Fig. 2. — Heptaclor sous-létal, avril. Aspect de dégénérescence du follicule prévitellogène. Détail : condensation du noyau de l'ovocyte (*N*), le cytoplasme raréfié (*C*), l'épithélium folliculaire désorganisé (*E*), cellules folliculaires — phagocytes (*CF*).
Fig. 3. — Heptaclor létal, avril. Germarium, détail : pycnoses (*p*), contraction des noyaux, tendance de raréfaction des cellules du germarium .
Fig. 4. — Heptaclor létal, avril. Follicule dégénéré au noyau comprimé.
Fig. 5. — Heptaclor létal, avril. Follicules prévitellogènes contractés (*F*).

PLANCHE I

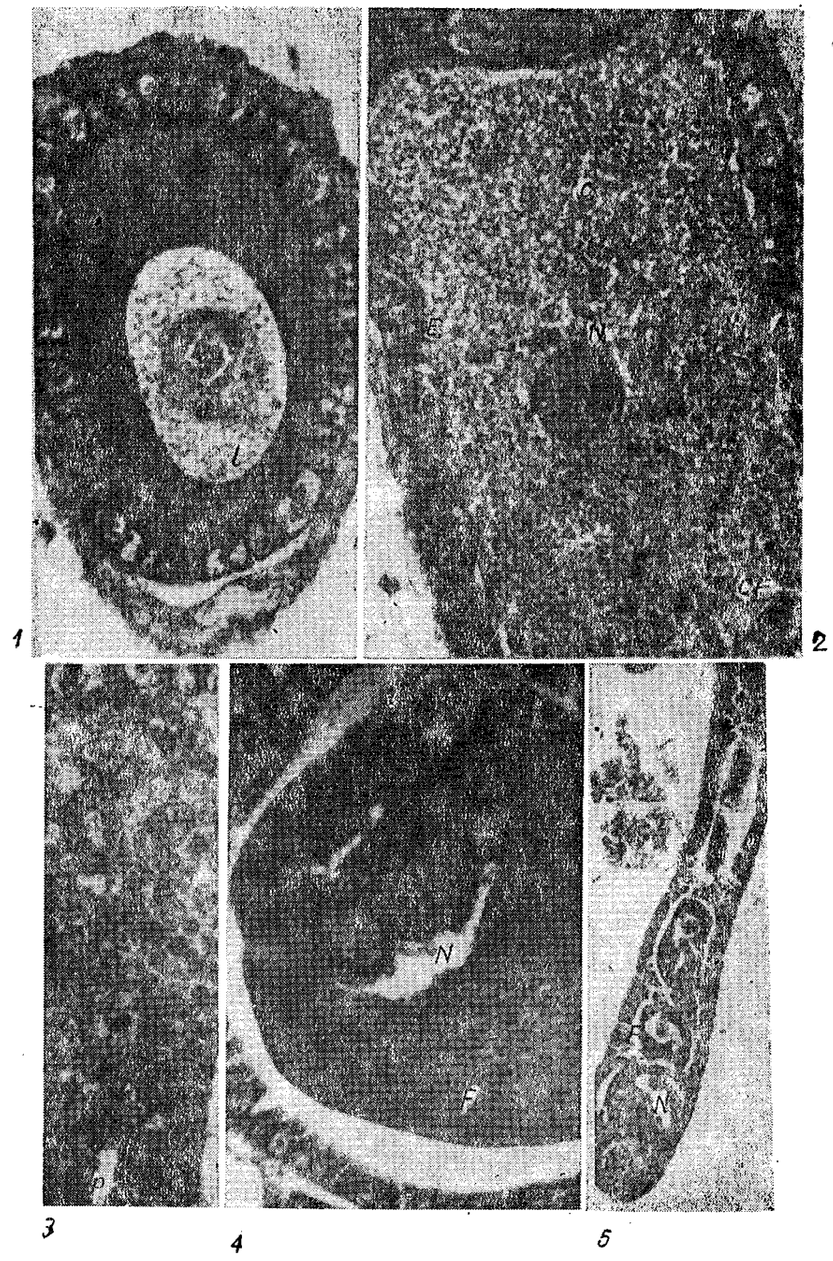
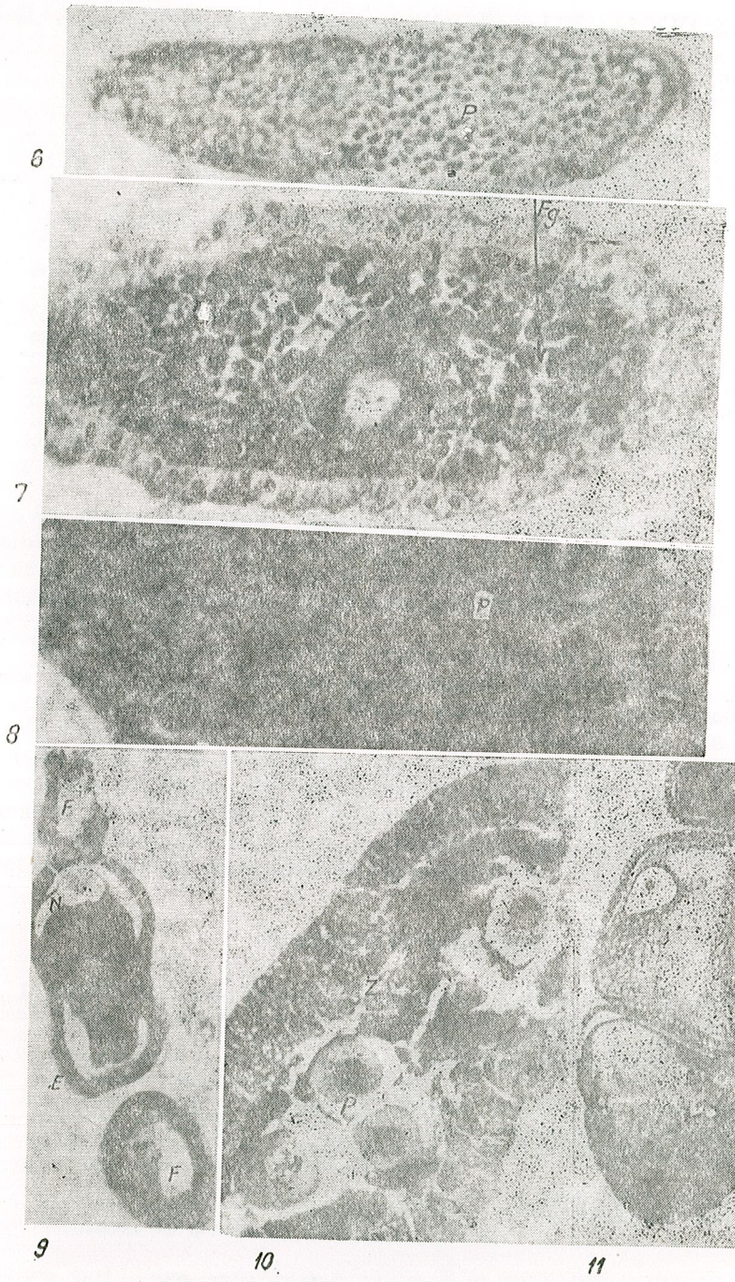


Fig. 6. — Heptaclor létal, avril. Germarium : nombreuses pycnoses (*P*).
Fig. 7. — Heptaclor létal, avril. Phagocytose avancée d'un follicule prévitellogène au noyau lésé. *Fg* = phagocytes.
Fig. 8. — Heptaclor sous-létal, mai. Germarium : pycnoses (*p*).
Fig. 9. — Heptaclor létal, mai. Follicule prévitellogène dégradé (*F*), noyau altéré (*N*). Epithélium folliculaire (*E*).
Fig. 10. — Fosfotox létal, mai. Follicules prévitellogènes dégénérés et amassés anormalement dans le vitellarium, pycnose (*p*). Epithéliums proliférés (*Z*).
Fig. 11. — Fosfotox létal, mai. Follicules vitellogènes dégénérés.

PLANCHE II



BIBLIOGRAPHIE

1. Bonnemaison L., 1969, C. R., Sci. Nat., **268**, 2078.
2. Forgash A. J., 1962, J. Ec. ent., **55**, 308.
3. Huignard J., 1971, C. R. Acad. Sci. Fr., **273**, 2557.
4. Manolache F., Văcaru A., 1972, Analele ICCS Braşov, **3**, 155.
5. Moriarty F., 1969, Biol. Rev., **44**, 321.
6. Ramade F., 1967, Ann. Inst. Nat. Agron., **5**, 1.
7. Ramade F., Rivière J. L., 1971, Ann. Soc. Ent. Fr., **7**, 709.
8. Ramade F., 1973, C. R. Acad. Sci. Paris, **276**, 985.
9. Rivière J. L., 1974, Bull. Soc. Zool. Fr., **99**, 121.
10. Teodorescu M., Trandaburu V., Văcaru A., 1974, Rev. Roum. Biol., **19**, 101.

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LE SPECTRE DE RÉSISTANCE AUX INSECTICIDES D'UNE SOUCHE ROUMAINE DE *MUSCA DOMESTICA* L.

PAR

JUSTIN GHIZDAVU et LETIŢIA GHIZDAVU

By laboratory tests (by topical and tarsal contact methods) were established the resistance levels to 16 of the most important insecticides of a Romanian strain (I.A.C.) of *Musca domestica* L. The strain is very sensitive to nicotine, DDVP, parathion, fenitrothion, dimethoate and mecarbam, sensitive to dipterex, disiston, malathion and ethion, moderately sensitive to DDT, lindane, aldrin and dieldrin and moderately resistant to toxaphene and sevin. Our strain is more resistant than the WHO strain and exhibits very different (superior, equal or inferior) levels of resistance to the different insecticides, comparatively to other foreign strains.

La masse corporelle assez importante, le cycle vital bref [5], la sensibilité modérée aux insecticides [9], la facile séparation des sexes [3] et la possibilité d'élevage en laboratoire [10] ont imposé la mouche domestique en toxicologie [1] et nous ont déterminé de la choisir comme principal test dans nos travaux.

Mais ne possédant pas une souche étudiée de ce point de vue, nous avons sélectionné, à l'Institut Agronomique de Cluj-Napoca, en partant d'une population indigène de *Musca domestica* L., une souche (I.A.C.) afin de l'employer dans nos futurs travaux de toxicologie. En supposant que celle-ci, grâce à un génotype spécifique, peut manifester par rapport aux souches étrangères un comportement différent envers les insecticides, pour avoir un système juste de référence pour nos résultats, nous avons déterminé les niveaux de résistance (de sensibilité) de cette souche envers les insecticides les plus importants et représentatifs.

MATÉRIEL ET MÉTHODES

La souche I.A.C. a ses origines dans une population présumée á avoir une sensibilité normale aux insecticides. Par des travaux de sélection pendant 20 générations, élevées en consanguinité constante (de type frère-sœur), nous avons obtenu une souche très homogène en ce qui concerne la taille (13—15 mg/♂ et 20—23 mg/♀) et le comportement envers les insecticides (vérifié par le χ^2 de l'erreur dans le cas de chaque test).

Nous avons déterminé les niveaux de résistance (respectivement de sensibilité) de notre souche envers 16 insecticides : nicotine, DDT, lindane, aldrin, dieldrin, toxaphène, dipterex (trichlorphon) DDVP (dichlorvos), parathion, fenitrothion, disiston, dimethoate (rogor), mecarbam, ethion, sevin (carbaryl), soigneusement purifiés.

Les niveaux de résistance ont été appréciés par les doses létales moyennes (DL-50), déterminées par la méthode des microgouttes (topique), et les concentrations létales moyennes (CL-50) déterminées par la méthode du contact tarsal [9].

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Dans le cas de la méthode topique, à l'aide d'un microapplicateur, on a déposé sur la partie dorsale du thorax des insectes, préalablement anésthésiés au CO_2 et pesés, des microgouttes de 4,398 µl de solution acétonique d'insecticide, la différenciation des doses se réalisant selon la concentration des solutions. Chaque dose a été appliquée à un lot de 100 individus placés dans des cages de verre et ayant à leur disposition de l'eau et de la nourriture. En partant de ces doses (rapportées à l'unité de masse corporelle) et des mortalités correspondantes, notées après 24, 48 et 72 heures, nous avons calculé selon l'analyse probit [5] les doses létales moyennes (DL-50).

Dans le cas de la méthode du contact tarsal les lots d'insectes ont été placés, sans eau et sans nourriture, sur des disques de papier filtre imprégnés d'insecticide de diverses concentrations (exprimées en µg/cm²), sous des couvercles de boîte de Petri. En marchant sur la surface traitée les insectes ont réalisé le contact avec la substance par les tarses. En partant de ces concentrations et des mortalités correspondantes notées après 12 heures dans le cas des mâles et après 24 heures dans le cas des femelles, nous avons calculé selon l'analyse probit [5] les concentrations létales moyennes.

RÉSULTATS ET DISCUSSIONS

Le tableau 1 présente les DL-50 et les CL-50 obtenus de nos expériences. Selon les DL-50, la souche I.A.C. apparaît comme très sensible envers la nicotine, le DDVP, le parathion, le fenitrothion, le dimethoate

Tableau 1

Les niveaux de résistance de la souche I.A.C. envers les insecticides étudiés

L'insecticide	DL−50 µg/g						CL−50µg/cm²	
	♀♀ après			♂♂ après			♀♀ après	♂♂ après
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	12 h
Nicotine	1,02	0,84	0,82	0,62	0,59	0,59	0,03	0,03
DDT	31,98	6,93	3,76	2,27	2,14	1,83	45,50	50,50
Lindane	38,59	20,39	17,50	20,17	20,08	11,60	9,80	2,01
Aldrin	36,18	32,07	25,03	7,39	6,81	6,40	22,10	30,50
Dieldrin	38,97	32,06	25,77	6,81	6,25	5,82	5,15	11,60
Toxaphène	174,75	132,53	109,70	28,82	22,79	21,22	57,50	52,00
Dipterex	12,87	12,87	12,37	3,18	2,97	2,86	0,28	0,10
DDVP	0,74	0,73	0,72	0,39	0,35	0,35	0,016	0,017
Parathion	1,40	0,69	0,40	0,50	0,24	0,19	0,05	0,006
Fenitrothion	3,46	2,24	1,45	1,46	0,93	0,93	0,06	0,06
Disiston	49,26	49,26	49,26	41,13	40,39	40,39	0,90	0,98
Dimethoate	1,99	0,53	0,23	1,22	0,52	0,38	1,08	0,88
Mecarbam	3,77	3,42	3,42	2,87	2,75	2,75	0,24	0,29
Malathion	17,67	10,73	10,24	1,04	1,04	1,04	2,33	2,23
Ethion	12,99	7,40	6,07	4,94	3,93	2,99	2,51	0,78
Sevin	120,24	90,34	90,34	21,34	20,26	19,47	49,00	45,50

et le mecarbam (DL-50 < 5 µg/g), sensible envers le dipterex, le malathion et l'ethion (DL-50 $= 5-15$ µg/g), modérément sensible envers le DDT, le lindane, l'aldrin, le dieldrin et le disiston (DL-50 $= 15-50$ µg/g) et relativement résistante au toxaphène et au sevin (DL-50 pour les femelles $= 100$ µg/g).

Les niveaux de résistance déterminés par la méthode du contact tarsal confirment généralement ceux exprimés par les DL-50. La souche se montre très sensible envers la nicotine, envers la majorité des organophosphates (dipterex, DDVP, parathion, fenitrothion, disiston, dimethoate et mecarbam) (CL-50 < 1µg/cm²) et un peu moins sensible au malathion et au ethion (CL-50 $= 1-5$ µg/cm²). Elle manifeste une sensibilité modérée aux insecticides organochloriques et au sevin (CL-50 $= 2-50$ µg/cm²).

Les tests réalisés par la méthode topique revèlent un manque de sensibilité envers le disiston et, dans les premières 24 heures, envers le DDT, situation explicable par le fait que le disiston est connu comme insecticide systémique et le DDT possède une faible action de choc [8].

Les différences de sensibilité observées dans le cas des divers insecticides et surtout dans le cas du disiston (en comparant les résultats obtenus par les deux méthodes) peuvent être expliquées par la différence de solubilité des substances dans le tégument au niveau de la partie dorsale du thorax (site de pénétration de la substance dans le cas de la méthode topique) et au niveau des pulviles des tarses (site de pénétration de la substance dans le cas de la méthode du contact tarsal). Cette solubilité différenciée peut être attribuée aux différences d'épaisseur et de structure du tégument. Elle joue un rôle très important dans le processus d'intoxication et représente une des principales causes du phénomène de sélectivité [12].

Tableau 2

Comparaison entre les niveaux de résistance des différentes souches

L'insecticide	DL−50 (µg/g) ♀♀ 24 heures							
	I.A.C.	a	b	c	d	e	f	g
DDT	31,98	75,00	4,03−75,45	—	—	0,13	8,0−21,0	—
Lindane	38,59	1,20	0,50− 2,09	—	—	0,01	2,00*	2,50
Aldrin	36,10	1,75	—	—	—	—	1,70	1,70
Dieldrin	38,97	1,50	11,26−13,48	—	—	0,013	1,30	1,30
Toxaphène	174,75	—	—	—	—	—	31,00	—
Dipterex	12,87	10,00	—	1,50	—	—	—	—
Parathion	1,40	0,75	—	1,40	—	—	—	0,90
Dimethoate	1,99	—	—	1,00	—	—	—	—
Malathion	17,67	—	3,54−26,84	12,00	27,00	0,57	—	28,00
Sevin	120,24	—	—	—	—	—	500,00	—

selon : a = Ramade (9); b = Millar et Hooper (6); c = Busvine (2); d = Townsend et Busvine (11); OMS/Millar et Hooper (6)/; f = O'Brien (7); g = Winteringham (12)

* = DL-50 calculée pour les mâles.

Le tableau 2 contient les niveaux de résistance de notre souche exprimés seulement par les DL-50 calculées pour les femelles à l'intervalle de 24 heures, comparés à ceux déterminés dans des conditions semblables, par d'autres auteurs, pour des souches étrangères. A l'égard du DDT, notre souche se montre plus sensible que la souche française de Ramade [9], elle se place entre les souches australiennes de Millar et Hooper [6], présentant en même temps une résistance supérieure à celle citée par O'Brien [7], et une résistance 246 fois supérieure à celle de la souche OMS. En ce qui concerne les autres insecticides organochloriques, la souche I.A.C. manifeste une résistance supérieure aux souches étrangères.

Quant au dipterex, notre souche est pratiquement égale à celle de Ramade [9], et plus résistante que la souche anglaise de Busvine [2].

A l'égard du malathion, notre souche présente une résistance inférieure à celle citée par Townsend et Busvine [11] et par Winteringham [12], elle se place entre les souches australiennes et est égale à la souche de Busvine [2], mais se montre plus résistante que la souche OMS. Envers le parathion, la souche I.A.C. montre une résistance supérieure à celle citée par Ramade [9] et par Winteringham [12] et une résistance égale à celle de la souche de Busvine [2]. En même temps, à l'égard du dimethoate, notre souche présente une résistance supérieure à celle de la souche de Busvine [2]. Enfin, quoique notre souche soit assez résistante au sevin, elle montre une résistance quatre fois moins importante que celle citée par O'Brien [7].

Il est évident que les résultats si variés obtenus par les divers auteurs ont des causes génétiques, dues à leurs différents génotypes.

Il résulte aussi que dans le cas de l'emploi de nouvelles souches en travaux de toxicologie, il est nécessaire de déterminer, avant tout, leur spectre de résistance aux insecticides les plus représentatifs, pour avoir un juste terme de comparaison.

BIBLIOGRAPHIE

1. Baicu T., 1966, *Culegere de metode utilizate în încercarea produselor fitofarmaceutice.*, Centr. Doc. Agr., București.
2. Busvine J. R., 1963, *Mededel. Rijks. Landbouw.*, Gent, **32**, *3*.
3. Dobreanu E., Berteanu A., Dumitreasa A., 1962, *Determinator al muștelor sinantrope din R.P.R.*, Ed. Academiei, București.
4. Fisher R. A., Yates F., 1963, *Statistical tables for biological, agricultural and medical research*, Oliver and Boyd, London.
5. Ghizdavu I., *Lucr. St. Inst. Agr. "Dr. P. Groza"*, 1975, Cluj-Napoca, **29**, 49–51.
6. Millar A., Hooper L. G. H.S., 1974, *J. Austral. Ent. Soc.*, **8**, 113–115.
7. O'Brien R. D., 1966, *Insecticides, Action and Metabolism*, Acad. Press. New York–London. 111, 136, 184.
8. Popa G., Drimuș R., 1961, *Chimia produselor fitofarmaceutice*, Ed. Tehnică, București, 61.
9. Ramade F., 1963, *Extr. Proc.-Verb. Acad. Agr. France*, 1062–1066.
10. Sawicki R. M., Holbrook D. V., 1961, *Pyrethrum Post*, **6**, *2*, 3–18.
11. Townsend M. G., Busvine J. R., 1969, *Ent. Exp. & Appl.*, **12**, 243–267.
12. Winteringham F. P. W., 1969, *Ann. Rev. Entomol.*, 409–443.

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ȘT. NEGREA, ALEXANDRINA NEGREA, **Ecologia populațiilor de cladoceri și gasteropode din zona inundabilă a Dunării** *(L'écologie des populations de Cladocères et Gastéropodes de la zone inondable du Danube), 232 p., 15 tableaux, 60 fig., résumé en français, Editions de l'Académie de la République Socialiste de Roumanie, 1975.*

Fruit d'un travail méticuleux sur deux groupes d'animaux à rôle essentiel dans le plancton et dans le benthon, ces recherches s'inscrivent dans l'effort déployé en vue de l'étude du complexe Crapina-Jijila, constituant sans doute l'étude de limnobiologie la plus rigoureuse et conséquente réalisée en Roumanie. Il n'existe aucun hiatus entre les parties analysant les facteurs du milieu et les parties biologiques. L'accent est mis sur l'analyse de la dynamique des populations. Le fondement théorique de l'ouvrage est constitué par la théorie systémique, suivant laquelle les populations sont des systèmes biologiques autoréglables, les recherches populationnelles permettant d'élucider les facteurs déterminant l'évolution et la productivité des biocénoses. Ceci — tout comme l'utilisation d'outils spécialement conçus pour l'échantillonnage d'un bon appareil statistique et de représentation graphique — permet aux auteurs de préciser, pour chacune des espèces de cladocères et de gastéropodes (dont on présente une classification écologique), les caractéristiques des populations considérées comme essentielles : répartition des individus dans l'espace, densité, croissance, structure suivant l'âge, les sexes, les dimensions. D'intéressants chapitres sont dédiés à la dynamique des associations de cladocères et de gastéropodes des eaux de différents types. Le travail se réfère à un milieu de vie probablement unique en Europe : la zone inondable d'un fleuve. Il a le mérite de souligner le rôle important des « Bălți » du Danube, de mettre en évidence l'impérieuse nécessité de protéger le complexe Crapina, qui doit devenir une réserve naturelle ouverte à l'économie piscicole et au tourisme.

L. Botoșăneanu

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