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FONTAINELLA MEDITERRANEA GEN. N., SP. N., CUMACÉ *(PSEUDOCUMIDAE)* TROUVÉ EN MÉDITERRANÉE ORIENTALE

PAR

MIHAI BĂCESCU et ZARUI MURADIAN

The authors, by studying a material collected in the waters of Libya, describe *Fontainella mediterranea* gen. n. sp. n. belonging to the family *Pseudocumidae*. Both sexes are figured.

On était incliné à considérer les *Pseudocumidae* — à quelques exceptions près — comme des cumacés caractéristiques de la faune relique, saumâtricole, du bassin pontocaspien, vu que 7 de ses 9 genres sont cantonnés là bas. Récemment, 1977, Ledoyer ajoute un nouveau genre océanique à cette famille, *Kerguelenica*.

Plus étonnant encore est le fait que l'on a dépisté, en étudiant le matériel capturé dans les eaux de Libye par l'équipe de l'Institut Roumain de Recherche Marine, conduite par le Dr G. I. Müller, un autre genre nouveau de cette famille, le genre *Fontainella*, que nous avons le plaisir de dédier au Professeur Maurice Fontaine, le Directeur de l'Institut Océanografique de Paris.

Fontainella gen. n.

Diagnose. Cumacé ayant des exopodites aux péréiopodes I et II seulement, donc trois pairs avec ceux du 3e Maxillipède (♂, ♀). Les deux paires de pléopodes du ♂ sont rudimentaires. A chaque articulation du flagelle de l'Antenne ♂, une botte d'aesthetascs d'une forme particulière.

Fontainella mediterranea n. sp.

(Figs. 1 et 2)

Matériel : 1 ♂ ad., 1 ♂ P., 1 ♀ ad. et 1 juv. (disséqué).

Provenance : Libye, St. 296, 57 m, en face d'El Hania, fond rocheux couvert d'algues, Août 1976 ; riche association de Nannastacidae.

Description ♂ adulte. Carapace comprimée dorso-ventralement ; sa largeur dépasse quatre fois sa hauteur et représente 1/3 de la longueur totale du crustacé (fig. 1 *A* et *B*). Pseudorostre si court que la partie frontale de la carapace présente une concavité. L'encoche antennaire

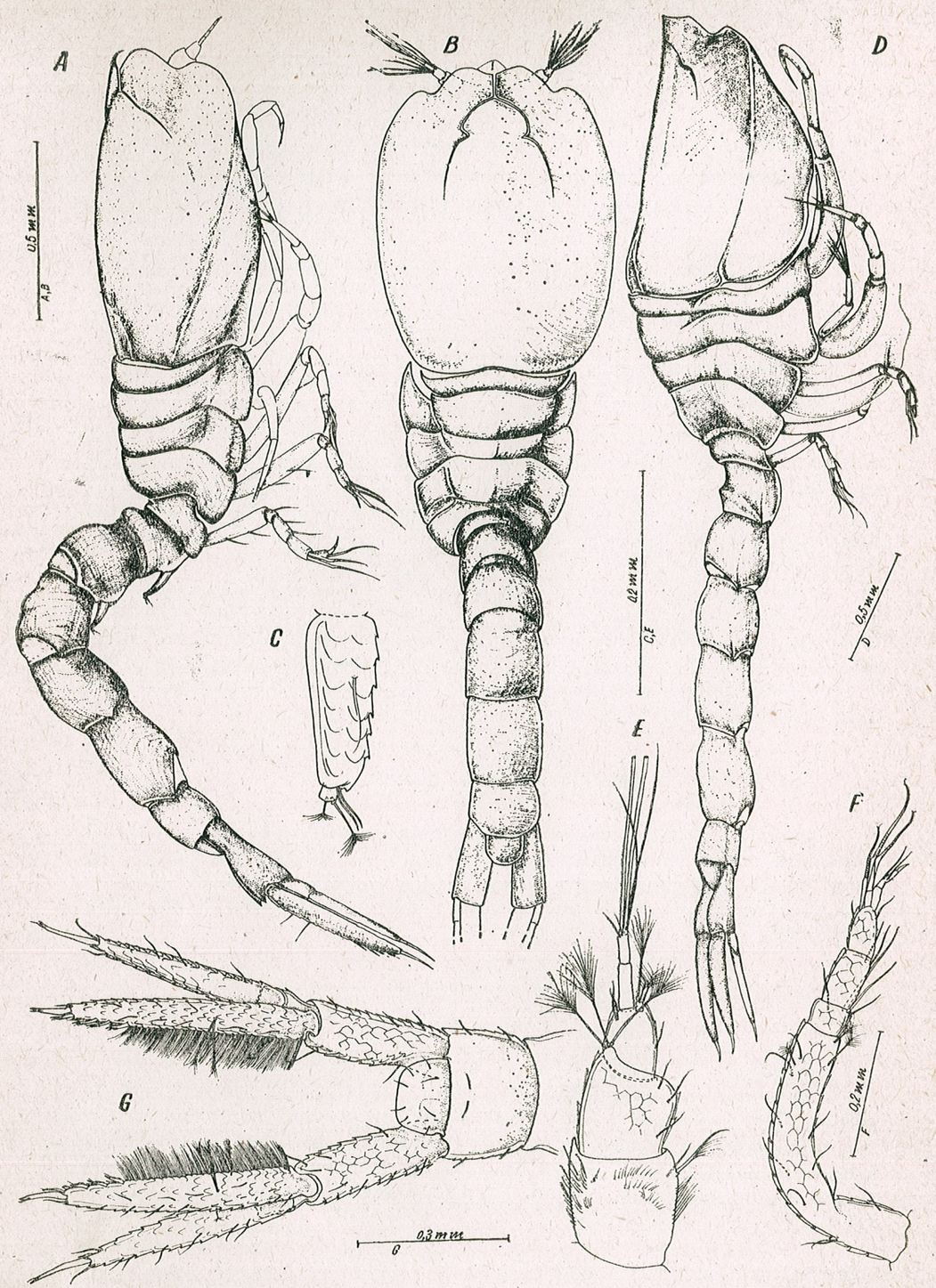

Fig. 1. — *Fontainella mediterranea* gen. n., sp. n. ♂ ad. *A*, *B*, vue de profil et d'en haut ; *C*, pléopode I. ♀ ad. ; *D*, vue de profil ; *E*, antennule ; *F*, péréiopode III ; *G*, dernier somite, telson et uropodes.

Fig. 2. — *Fontainella mediterranea* gen. n., sp. n. ♂ ad. *A*, antennule ; *B*, antenne ; *C*, mandibules ; *D*, seconde maxille ; *E*, maxillipède I ; *F*, maxillipède II ; *G*, maxillipède III ; *H*, péréiopode I ; *I*, péréiopode II ; *J*, péréiopode III ; *K*, péréiopode V ; *L*, dernier somite, telson et uropodes. ♀ ad. ; *M*, antenne.

faiblement marquée et l'angle antero-latéral, arrondi. Lobe oculaire cordiforme et sans éléments visuels. Le II^{e} péréionite est le plus élargi.

Le telson a la forme d'une lame en demi-cercle, ornée seulement de quelques courtes soies. Antenne I (Fig. 2 *A*) présente une grande richesse d'aesthetascs. II^{e} Antenne (fig. 2 *B*) avec le 5^{e} article du pédoncule égalant en longueur les autres considérés dans leur ensemble; son flagelle a 17 articles, chacun d'eux ayant une botte de 3 à 5 aesthetascs d'un type spécial: courtes et finissant brusquement en pointe. On voit la morphologie de la mandibule et de la II^{e} maxille sur les figures 2 *C* et *D*.

Le I^{er} maxillipède (fig. 2 *E*) présente sur le bord inféro-interne du carpe une espèce à part de phanères, pectinés. Sur les figures 2 *F* et *G* on voit les maxillipèdes II et III; ils ont les basis subégaux aux restes des appendices. Le basis du I^{er} péréiopode (fig. 2 *H*) n'atteint qu'une moitié de l'appendice. La II^{e} patte est plus courte que la première (fig. 2 *I*). Les autres péréiopodes (fig. 2 *J* et *K*) ont des dactylopodites très courts, largement dépassés par les soies articulées et flagelées des derniers articles. Pas d'exopodites aux péréiopodes III et IV. Les pléopodes I et II (fig. 1 *C*) sont réduits à de courts pédoncules, surmontés d'un minuscule article sur lequel s'insèrent 2 soies sensitives. Le pédoncule uropodal (fig. 2 *L*) dépasse à peine le dernier somite et il est bien plus court que les rames; l'endopodite a une dense garniture de cils fins rappelant la même structure chez *Pseudocuma ciliata*.

Taille: 2,6 mm.

Description ♀ *adulte*. Le céphalothorax ressemble à celui du ♂, étant pourtant plus haut, sa longueur représente 1/3 du corps (fig. 1 *D*). Pour la première Antenne, voir fig. 1 *E*. L'Antenne II finit par une soie très forte et pectinée (fig. 2 *M*). Les pattes de la 3^{e} et 4^{e} paire (fig. 1 *F*) sans exopodites. Les uropodes (fig. 1 *G*) ont des pédoncules plus courts qu'une moitié de la longueur des rames.

Taille: 3,8 mm—du rostre au bout du telson.

Holotype ♂ adulte, déposé dans la collection du Musée d'Hist. nat. «Gr. Antipa» sous le N° 407; Allotype, ♀ adulte, ibid, N° 408 et un Paratype ♂ subadulte, ibid N° 407 b.

Discussion. A première vue, nos Cumacés semblent appartenir au genre *Kerguelenica*. Mais une comparaison avec la minutieuse description de Ledoyer — et même sans pouvoir comparer les mâles (pas connu pour le *Kerguelenica*) — le fait qu'au g. *Fontainella* manquent les exopodites aux III^{e} et IV^{e} pattes et que son telson n'est pas triangulaire, exclut tout rapprochement.

Le nombre réduit des exopodites chez les deux sexes, de même que la réduction des pléopodes I et II chez le ♂, justifient amplement la création de ce nouveau genre, en enrichissant ainsi la famille des Pseudocumidae d'une nouvelle unité océanique.

BIBLIOGRAPHIE

1. BĂCESCU M., 1950, *Cumacei mediteraneeni modificaţi de mediul pontic . . .*, Anal. Acad. R.P.R., Sect. Geol. Geogr., **3**, *11*, 423—459.
2. LEDOYER M., 1977, *Cumacés (Crustacea) des Iles Kerguelen recueillis par le N. O. «La Japonaise» en 1972 et 1974 . . .*, CNFRA, **42**, 193—213.
3. LOMAKINA N. B., 1958, *Kumovîie raki (Cumacea) Morei SSSR*. Izd. Akad. Nauk, Moskwa, **66**, 1—302.
4. SARS G. O., 1900, *An account of the Crustacea of Norway*, **3**, III; *Cumacea*. Bergen, 1—115.

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EPICRIIDAE FROM THE ROMANIAN FAUNA

BY

LIBERTINA SOLOMON

The paper describes the first two species of *Epicriidae* from the Romanian fauna : the female, male and protonymph of *Epicrius kargi* n. sp., and notifies the presence of *Epicrius resinae* Karg 1971 ; new for Romania.

The representatives of the family *Epicriidae* Berlese, 1885, soil mites of the cohorts *Epicriina* (*Mesostigmata*) have been known in Europe, especially in the west and central parts and in Japan. Lately, besides numerous European species, some new ones have been described in U.S.S.R. (Bregetova, 1977).

Between 1975 and 1977, a complex synecological study drawn out from two forest ecosystems, one on the Moldavian Plateau (the Voinești—Iași forest, 1975—1976) and the second in the northern mountainous region of Moldavia (the Slătioara—Rarău secular forest 1977) led to the collection of a rich acarological material. Two representatives of the *Epicriidae* family were identified for the first time in our country, in these areas : one of them a new species, *Epicrius kargi* (dedicated to the acarologist W. Karg), and the other one, *Epicrius resinae* Karg, 1971, new for the Romanian fauna.

Epicrius kargi n. sp.

Female : The dorsal shield, strongly sclerotized, is 409—472 μ long × 305—352 μ wide (Fig. 1). It is provided with an areolate net built up of ovale, bi-or trifurcate tuber, and 31 pairs of fine pilose setae. Their length is slightly increased to the posterior end (33—48—64 μ), except for the setae r_1—r_5, small (7—9 μ) and simple. Between setae s_7—S_1 there are two dorso-lateral organs, typical for the genus. In the middle of the dorsal shield, a transverse double suture with a soft tegument is noticeable. The postero-lateral sides of the dorsal shield are bent on its ventral side, covering 1/3 of the opisthogastric surface.

On the ventral side (Fig. 2), the tritosternum has a long basis and two short tritined laciniae. There are two anterior small jugular shields bearing St_1 setae (14—24 μ). Setae St_2 and St_3 (26—31 μ) are placed on the sternal shield, which is trapezoidal (29—38 μ long × 60—67 μ wide) concave on its posterior edge. Setae Mst are placed on the tegument. The genital tronconic shield (143—155 μ × 114—131 μ) bears two pairs of setae (24—31 μ). There is one pair of setae on the tegument between the genital and anal shields. The anal shield, trapezoidal, (86—100 μ × 74—90 μ) is free and has a pair of praeanal setae, out of

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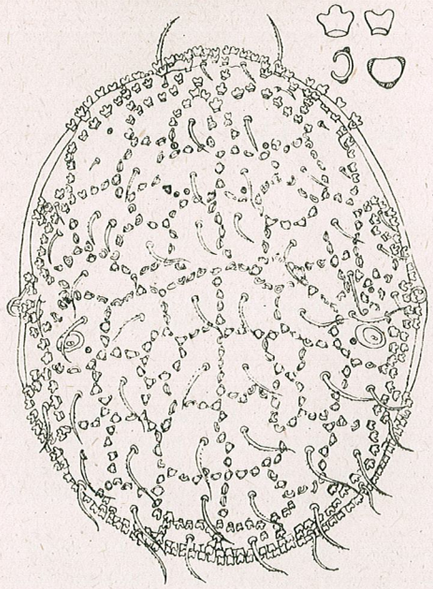

Fig. 1. — *Epicrius kargi*, female, dorsal.

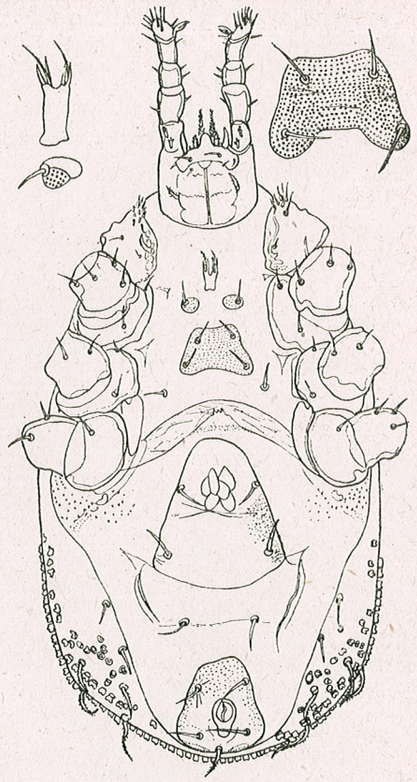

Fig. 2. — *Epicrius kargi*, female, ventral.

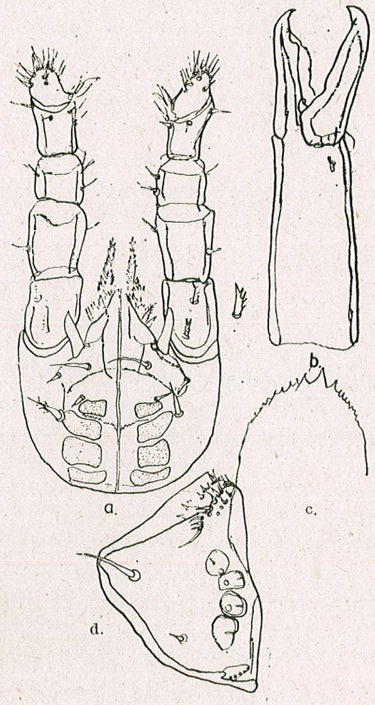

Fig. 3. — *Epicrius kargi*, female : *a* = gnathosoma ; *b* = chelicera ; *c* = tectum ; *d* = coxa I.

the anal setae (31—40 μ), disposed behind the anus. The end dopodal shields are small, like weak sclerotized sticks, placed between coxae I—II, II—III. The stigmae without peritreme, are placed on the coxae IV level. Four pairs of setae are on the curved sides of the dorsal shield.

All the shields present partial or total granular sculpture.

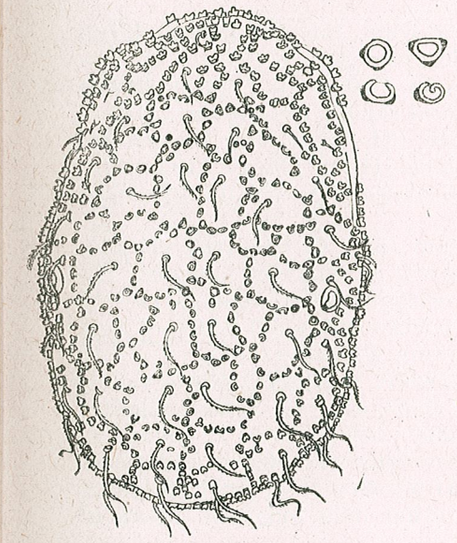

Fig. 4. — *Epicrius kargi*, male, dorsal.

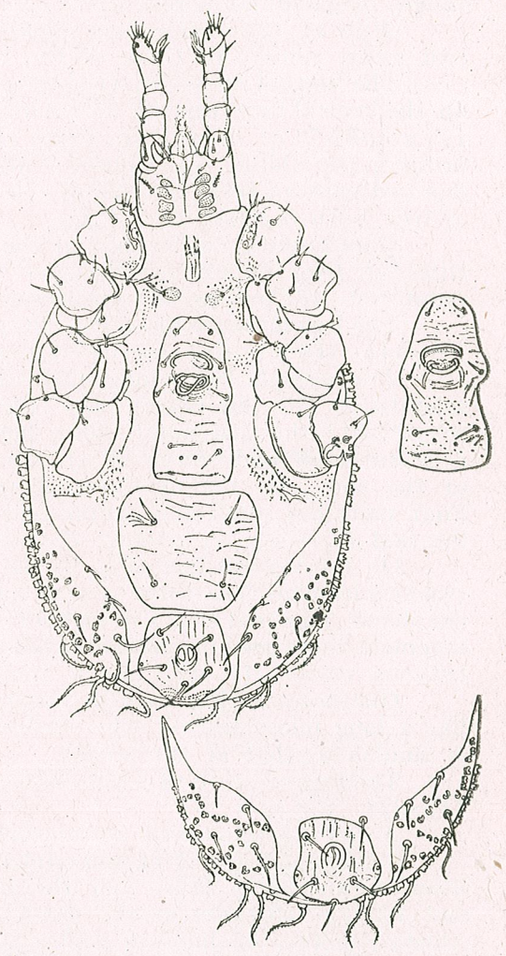

Fig. 5. — *Epicrius kargi*, male, ventral.

Gnathosoma (Fig. 3 a) 86—90 μ long × 79—93 μ wide, is provided with a single transverse denticulate line (Q). The corniculi are distinctly stalked, and the hypostomal processes are strong pennate. The gnathosomal setae (17 μ) are weakly pilose, and the hypostomal are simple, measuring : hy_1=26—31 μ, hy_2=7 μ, hy_3=9,5 μ. The palps (121—131 μ) have a long apotel ; the two palpal trohanters setae are comblike. The fingers of the chelicerae (Fig. 3 b) have each of them a small tooth. The tectum (Fig. 3 c) is convex, with a delicate denticulate edge.

The legs measure: I=609—619 μ, II=380—428 μ, III=357—381 μ, IV=433—476 μ. The first leg is thin, with long tibia (105—114 μ) and tarsus (143—152 μ), the tarsus being provided with acicular setae. Coxa I (Fig. 3 d) has a trimming of sensitive setae in the anteroinner corner, four ovale impressions on the gnathosomal side, and posteriorly 1—2 ranges of glandular pores.

Male of 428—447 μ long×286—309 μ wide, has the dorsal shield and setae, similar to those of the female (Fig. 4).

The sternal setae St_1 (17—21 μ) are on two small jugular shields on the ventral face (Fig. 5). The sterno-genital shield, rectangular, (133—155 μ×67—79 μ) bears four pairs of setae (24—26 μ) and the genital orifice in the middle. The square ventral shield (100—112 μ×119—129 μ) has two pairs of setae (24—26 μ). The anal shield (76—86 μ×76—86 μ) is free, with 5 setae (26—36 μ).

Gnathosoma, chelicerae and the tectum (Fig. 6) have the peculiarities of the species. Gnathosoma is 76—81 μ long×88 μ wide, the palps 102—131 μ long, the chelicerae are without spermatodactyl.

The legs measure: I=580—590 μ, II=362—414 μ, III=362—376 μ, IV=447—466 μ; tibia I=100—112 μ, Tarsus I=143—155 μ.

Protonymph, 290 μ long×228—233 μ wide, is slightly white-yellow sclerotized. On the dorsal face (Fig. 7) there are two shields slightly sclerotized: the podonotal (200—209 μ×205—209 μ) with 22 setae, i_4, i_5 setae small and simple (8 μ), the others long (42—62 μ) and pilose at the end, and the opisthonotal (76—81 μ×36—38 μ) with 8 pairs. Four pairs are small and simple and the other ones long and pilose at the end (I_1=71 μ, I_5=100 μ, S_1=86 μ).

On the ventral face (Fig. 8) the tritosternum has a long basis and two long laciniae. The three sternal setae are on a hardy noticeable sternal shield. The anal shield is without preanal setae. On the opisthogastric tegument there are four pairs of setae and an understigmatic seta. Stigma, between coxae III—IV, has a short peritrema.

Gnathosoma (Fig. 9) with simple setae and the hypostomal process like a long pilose tongue. The chelicerae are long and thin, the tectum is similar to that of the adults.

The legs measure: I=238—252 μ, II=190—205 μ, III=190—195 μ, IV=228—233 μ.

A few nymphal and larval stages of this family are known.

8 ♀♀, 5 ♂♂ and 3 protonymphs of this species were found in the litter, fermentation layer and the terricolous moss synusia, at 860 m and 1 120 m altitude, in the Slătioara—Rarău secular forest (Picето-Abieto-Fagetum oxalidosum association and Picetum carpathicum); 1 ♀, 1 ♂ was found in the litter and fermentation layer in the Voinești—Iași forest (Fageto-carpinetum association) at 150—402 m altitude.

The female has some similarities with *Epicrius ivanovi* Bregetova 1977, concerning the dorsal shield and setae structure, but is different from the latter, in the ventral structure which is closer to *E. tauricus* Bregetova 1977 and *E. pinetorum* Bregetova 1977. The distribution of ventral and anal setae on the two shields in male is similar to *E. monticola* Bregetova 1977.

The holotype and paratypes are in the collection of the author.

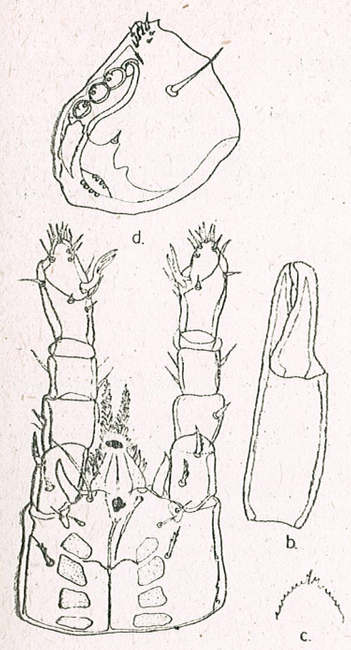

Fig. 6. — *Epicrius kargi*, male: *a*=gnathosoma; *b*=chelicera; *c*=tectum; *d*=coxa I.

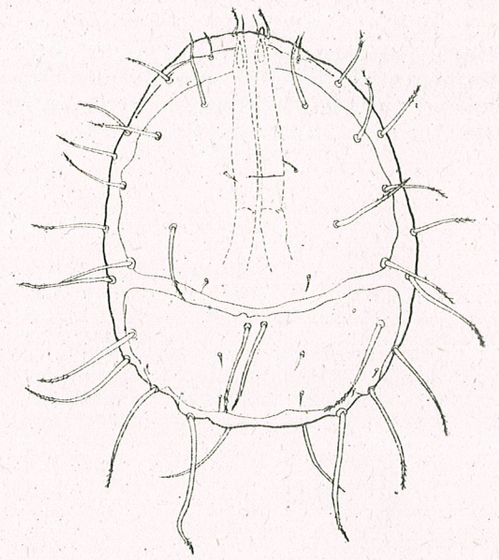

Fig. 7. — *Epicrius kargi*, protonymph, dorsal.

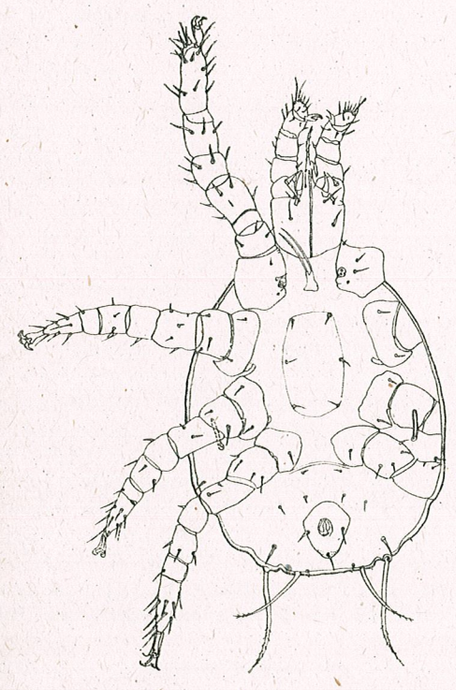

Fig. 8. — *Epicrius kargi*, protonymph, ventral.

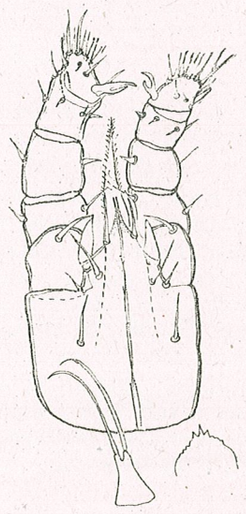

Fig. 9. — *Epicrius kargi*, protonymph, gnathosoma.

Epicrius resinae Karg, 1971.

T1 ♀♀ and 2 ♂♂ from this species were found in the Slătioara—Rarău secular forest on May 13, 1977 and July 5, 1977, in the litter, fermentation layer, saproxylicolous medium and moss. This species is known only in the German Democratic Republic where it was described for the first time and in U. S. S. R.

REFERENCES

1. Athias-Henriot C., 1961, Acarol. **III**, *4*, 381—509.
2. Ghiliarov M. S. et colab., 1977, *Opredeliteli obitayushchihh v pochive kleshchey Mesostigmata* Izd. Nauka, Leningrad.
3. Karg W., 1971, Zool. Anz., Leipzig **186**, *1/2*, 105—114.
4. Schweizer J., 1961, *Die Landmilben der Schweiz* (*Parasitiformes Reuter*). Gebrüder Fretz, Zürich.

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LES ÉCAILLES ANDROCONIALES CHEZ LES *POLYOMMATINAE (LYCAENIDAE)* — BONS CRITÈRES SPÉCIFIQUES ET GÉNÉRIQUES

PAR

EUGEN V. NICULESCU

The author presents the androconial scales in 29 species (19 genera), of *Polyommatinae* (*Lycaenidae*). After the description of these scales' structure is carried out, one of their peculiar features, namely the intraindividual variation proper only to the scales, as stated by the author, is emphasized. This variation is very important in most species, a peculiarity which determined the author to find out three scale categories: dominant, variant and aberrant ones. The specific characters are given by the dominant scales and may be evidenced only if a large number of scales is examined and the amplitude of the intraindividual variation is established. If these recommendations are carefully followed, it may be seen that the androconial scales show proper specific and generic characters which may be utilized in taxonomy.

Les écailles androconiales chez les *Polyommatinae* sont répandues parmi les écailles pigmentaires sur la face supérieure des ailes. Elles se trouvent chez les mâles seulement, tant sur les ailes antérieures que sur les postérieures.

Ces écailles ont des formes différentes. La plupart sont ovales, plus larges ou plus étroites, selon les espèces. Quelques unes sont sphériques (fig. 1,*5*) ou presque sphériques (fig. 1, *3*), comme une coupe fig. (1,*2*), ou en forme de coeur (fig. 1, *23*), de poire (fig. 1, *14*), de fuseau (fig. 1, *31*, *35*), etc. Parfois les bords latéraux sont droits (fig. 1, *4*, *8*, *9*, *10*), mais le plus souvent ces bords sont plus ou moins convexes (fig. 1, *5*, *7*, *14*, *18*, *19*, *35*, etc.). Chez certaines espèces elles sont plus larges vers le pôle supérieur (fig. 1, *14*, *17*, *23*, *30*), chez d'autres, au contraire, sont plus larges vers le pôle inférieur (fig. 1, *29*, *32*, *34*).

Les écailles androconiales sont dépourvues de sinus, mais le pédoncule est toujours présent. Sur leur surface on peut observer un dessin qu'en tracent les costulae longitudinales et les nodules formant des rangées transversales. Les costules, généralement bien visibles et parallèles, ont un trajet complet d'un pôle à l'autre (fig. 1, *22*, *23*), mais il arrive souvent qu'elles soient incomplètes (fig. 1, *10*, *19*, *21*, *27*, *30*, *32*) ou que leur trajet soit dévié (fig. 1, *29*, *34*). Parfois les costules sont invisibles ou presque — quoique les rangées longitudinales de nodules soient régulières (fig. 1, *3*, *18*, *25*). Quand les costules ont un trajet dévié ou sont interrompues (fig. 1, *5*, *30*, *32*, *33*) le dessin est irrégulier. Parfois les costules sont très fines (fig. 1, *8*, *9*, *19*, *20*, *36*), parfois elles sont plus grosses

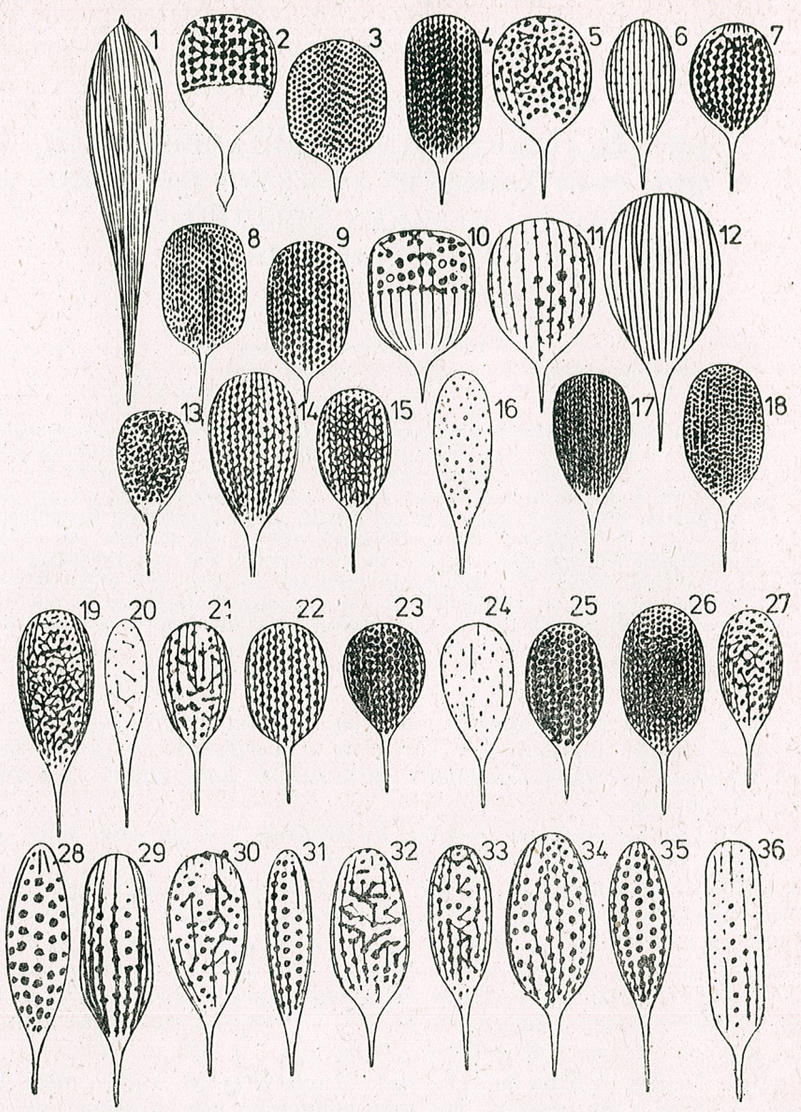

Fig. 1. — *1, Lampides boeticus* L.; *2, Syntarucus pirithous* L.; *3, Everes argiades* Pallas; *4, Cupido minimus* Fuessly; *5, Glaucopsyche alexis* Poda; *6, idem,* écaille aberrante; *7, G. melanops* Bsdv.; *8, Tarucus theophrastus* F.; *9, E. alcetas* Hoffm.; *10, Celastrina argiolus* L.; *11, G. alexis* Poda, écaille aberrante; *12, idem*; *13, Maculinea arion* L.; *14, M. alcon* Den. et Schiff.; *15, M. teleius* Bergstr.; *16, idem,* écaille aberrante; *17, Iolana iolas* Ochs.; *18, Philotes baton* Bergstr; *19, Plebejus pylaon* Fisch. *20, idem,* écaille aberrante; *21, P. argus* L.; *22, Lycaeides idas* L.; *23, Vacciniina optilete* Knoch; *24, idem,* écaille aberrante; *25, Albulina orbitulus* de Prunner; *26, Agriades glandon* de Prunner; *27, Cyaniris semiargus* Rott.; *28, Agrodiaetus dolus* Hbn.; *29, A. damon* Den. et Schiff.; *30, Polyommatus escheri* Hbn.; *31, P. dorylas* Den. et Schiff.; *32, P. amandus* Schneider; *33, P. thersites* Cant.; *34, Meleageria daphnis* Den. et Schiff.; *35, Polyommatus icarus* Rott.; *36, idem,* écaille aberrante.

(*A. damon* (fig. 1, *29*)), ou, chez certaines écailles, aberrantes (*P. coridon*, *P. bellargus*, etc.) Le nombre des costules est variable individuellement et spécifiquement. Le plus petit nombre de costules observé par nous a été de 3, 4 ou 5 (*P. icarus*[1]), ou 4 (*P. thersites*, *A. dolus*). Des costules nombreuses se trouvent chez *M. alcon* (13—16), *E. argiades* (15—19), *Ph. baton* (20—23), etc.

Les nodules sont enfilés sur les costules comme les rassades sur une ficelle. Leur forme est variée : sphérique, ovale, rectangulaire ou, très souvent, irrégulière. Ils sont tantôt indépendants l'un de l'autre (fig. 1, *28*, *31*, *34*, *35*) tantôt contigus (fig. 1, *3*, *4*, *7*, *18*, *26*), le nombre des nodules qui se touchent étant variable selon les espèces. Chez certaines espèces les nodules sont réunis par des stries courtes et fines, formant ainsi un réseau qui peut être lâche (fig. 1, *2*, *5*, *10*, *14*, *21*, *27*, *30*, *33*) ou dense (fig. 1, *13*, *15*, *19*, *26*). Les nodules forment des rangées longitudinales au long des costules sur lesquelles ils sont disposés, soit régulièrement (fig. 1, *4*, *14*, *22*) soit irrégulièrement (fig. 1, *5*, *15*, *19*, *27*, *30*, *32*). Parfois les costules ne sont pas visibles et toutefois les nodules forment des rangées longitudinales (fig. 1, *3*, *18*). Ils forment aussi, en même temps, des rangées transversales, le plus souvent irrégulières, parfois plus ou moins régulières, c'est-à-dire parallèles entre elles. Comme variantes intraindividuelles nous avons rencontré, chez *P. coridon*, un dessin très régulier, où les nodules sont liés les uns aux autres tant longitudinalement que transversalement, en formant ainsi des carreaux réguliers.

Les nodules peuvent être comptés, mais cette opération est assez difficile vu que les rangées transversales sont le plus souvent irrégulières. Nous avons imaginé une formule du dessin qui comprend le nombre des costules (C) et le nombre des nodules (N) ; en multipliant les deux nombres nous obtenons le nombre total des nodules — nombre, nous le répétons, approximatif. Ainsi la formule de *P. argester* est : C 5, N 12 ; *L. idas* : C 11, N 12 ; *V. optilete* : C 13, N 14 ; *C. minimus* : C 15, N 19 ; *Ph. baton* : C 19, N 26, etc. Le nombre total des nodules dépend de la largeur et la longueur de l'écaille. Le nombre le plus petit de nodules observé par nous a été de 14 chez certaines écailles de *A. dolus*, où les nodules se trouvaient seulement sur 2 costules. A l'extrême opposé se trouve *Tarucus teophrastus*, *Agriades glandon*, *Philotes baton*, etc. ; chez une écaille de cette dernière espèce nous avons compté 494 nodules.

Le nombre, la forme et les dimensions des nodules reflètent la structure interne des écailles. La variation du dessin intraindividuelle et interspécifique, nous montre combien variée et compliquée est cette structure, puisque les nodules ne sont pas autre chose que les points d'insertion des « pilons » (trabecules) qui réunissent la face supérieure et celle inférieure de l'écaille. Les écailles de certaines espèces (*L. boeticus*) sont dépourvues de nodules ; ce caractère spécifique est, chez d'autres espèces, aberrant. Nous avons observé de telles écailles aberrantes chez *G. alexis* (12), *P. coridon caelestissima*, *P. icarus*, *P. thersites*, *P. argester* et *P. amandus*. Chez toutes ces espèces de *Polyommatus* l'écaille aberrante a la même longueur et largeur et toujours 11 costules.

[1] L. G. Higgins et N. D. Riley [2] affirment que chez *icarus* les androconies manquent ; cette affirmation est erronée.

Parmi les nodules se trouvent les espaces internodaux qui peuvent être réguliers ou irréguliers, larges ou réduits. Le plus souvent ils sont irréguliers puisque les rangées transversales de nodules sont, elles aussi, irrégulières (fig. 1, *2*, *5*, *10*, *15*, *19*, *21*, *27*, *30*, *32*).

Les écailles androconiales chez les *Plebejinae* présentent la particularité d'être en grand nombre chez le même individu. Sur une lame frottée avec l'aile d'un papillon mâle se trouvent quelques centaines d'écailles androconiales et un nombre beaucoup plus grand d'écailles pigmentaires. Les écailles androconiales existantes sur une seule préparation d'un seul spécimen sont extrêmement variées ; cette variation nous l'avons nommée *variation intraindividuelle*, terme nécessaire pour la distinguer de la variation individuelle habituelle — d'un individu à l'autre — (variation interindividuelle). Nous précisons que la variation interindividuelle d'une même espèce n'est pas plus grande que celle intraindividuelle.

En examinant un grand nombre d'écailles sur une préparation, nous constatons presque toujours une grande variation intraindividuelle, mais cette variation ne dépasse pas certaines limites. A l'interieur de ces limites nous pouvons établir le caractère spécifique si nous tenons compte de l'amplitude de la variation intraindividuelle, y compris celle des écailles aberrantes. Nous avons constaté que chez une espèce quelconque le caractère spécifique est fourni par les *écailles dominantes* qui existent en proportion de 70 —80%. Parmi ces écailles s'en trouvent d'autres, moins nombreuses (20 — 30%) mais très variables, auxquelles nous avons donné le nom d'*écailles variantes* ; ce sont les écailles qui nous indiquent l'amplitude de la variation intraindividuelle. Enfin on observe presque toujours 1—2 écailles de structure insolite, nommées « écailles aberrantes « (fig. 1, *6*, *11*, *12*, *16*, *20*, *36*). Des considérations ci-dessus résulte la necéssité d'examiner un grand nombre d'écailles — opération d'ailleurs très facile — pour constater les caractères spécifiques. Si, d'une part, nous notons avec soin les particularités des écailles variantes et établissons les limites de la variation intraindividuelle et d'autre part nous observons toujours avec soin les écailles dominantes, nous pouvons trouver aisément les caractères spécifiqes et séparer les espèces l'une de l'autre. Une étude attentive effectuée et interprétée d'une façon juste, nous montre que les écailles androconiales chez les *Polyommatinae* diffèrent d'une espèce à l'autre et nous offrent en même temps des caractères génériques utiles pour la taxonomie.

Cette étude nous a montré que l'affirmation de M. Guillaumin et H. Descimon [1] selon lesquelles, « les androconies se sont révélées finalement absolument trompeuses» ne peut être retenue. Nous devons admettre que les androconies fournissent de bons caractères spécifiques et génériques si elles sont examinées minutieusement selon nos indications.

Voici maintenant les caractères spécifiques des écailles androconiales chez quelques *Polyommatinae*.

Lampides boeticus (fig. 1, *1*) écailles fusiformes dépourvues de nodules.

Syntarucus pirithous (fig. 1, *2*). C 10, N 7. Dessin localisé seulement dans la moitié supérieure de l'écaille.

Everes argiades (fig. 1, *3*). C 15—19, N 14—12. Ecailles sphériques ou avec les bords droits, costules invisibles.

Cupido minimus (fig. 1, *4*). C 15, N 19. Les bords latéraux droits, les costules fines.

Glaucopsyche alexis (fig. 1, *5*). Ecailles sphériques, dessin très irrégulier, les nodules forment un réseau rare.

G. melanops (fig. 1, *7*). C 11, N 12. Ecailles presque sphériques, les costules regulières, le réseau rare, localisé seulement dans la partie supérieure de l'écaille.

E. alcetas (fig. 1, *9*). C 14, N 18. Les costules fines, un réseau rare au milieu de l'écaille.

Celastrina argiolus (fig. 1, *10*). Ecailles en forme de coupe. Les costules, qui se trouvent seulement dans la moitié inférieure de l'écaille, sont régulières ; les nodules, localisés dans la moitié supérieure, forment un dessin irrégulier.

Maculinea arion (fig. 1, *13*). Le dessin irrégulier forme un réseau très dense.

M. alcon (fig. 1, *14*). C 13, N 17. Ecailles pyriformes, les costules régulières, les nodules forment un réseau rare.

M. teleius (fig. 1, *15*), C 12, N 18. Le réseau plus dense que chez alcon, le pédoncule plus long et plus mince.

Philotes baton (fig. 1, *18*). C 19, N 26. Les nodules très nombreux, les costules invisibles, mais par endroits elles sont assez grosses, courtes, réunissant plusieurs nodules.

Plebejus pylaon (fig. 1, *19*). Le dessin très irrégulier, le réseau assez dense.

Lycaeides idas (fig. 1, *22*). C 11, N 12. Le dessin très régulier.

Vacciniina optilete (fig. 1, *23*). C 13, N 14. Ecailles cordiformes, les nodules grands, les espaces internodaux très réduits.

Agrodiaetus dolus (fig. 1, *28*). Ecailles fusiformes avec quelques costules incomplètes à la partie supérieure.

A. damon (fig. 1, *29*). Les costules plus nombreuses, certaines avec trajet dévié.

Polyommatus escheri (fig. 1, *30*). Le dessin très irrégulier, le réseau très rare.

P. dorylas (fig. 1, *31*). Ecailles fusiformes, 5 costules dont deux incomplètes, sans réseau, le pédoncule court.

P. amandus (fig. 1, *32*). Ecailles plus larges à la partie postérieure, le dessin très irrégulier, le réseau plus dense que chez *P. escheri*.

P. icarus (fig. 1, *35*). C 5, N 13. Ecailles fusiformes, plus larges que celles d'argester, le pédoncule long.

BIBLIOGRAPHIE

1. Guillaumin M., Descimon H., 1976, *La notion d'espèce chez les Lépidoptères*, in *Les problèmes de l'espèce dans le règne animal*, Ed. Soc. Zoologique de France, I, 129—201.
2. Higgins L. G., Riley N. D., 1971, *Guide des papillons d'Europe*, Ed. Delachaux et Niestlé, 414.
3. Niculescu E. V., König Frederic, *Fauna Republicii Socialiste România. Lepidoptera. Partea generală*, **IX**, *10*, 3—300.
4. Verity Ruggero, 1943, *Le farfalle diurne d'Italia, Fam. Lycaenidae*, **II**, 404.

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L'INFLUENCE DES GLANDES CORPORA ALLATA DE *GRYLLOTALPA GRYLLOTALPA* LATR. SUR LA GONADE FEMELLE DANS DES CULTURES *IN VITRO*

PAR

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The corpora allata of adult *Gryllotalpa* contain light and dense glandular cells and hypertrophic multinuclear formations. After conservation *in vitro* the activity of the light cells decreases as a consequence of the corpora allata alterations, though the connections of these glands with the neuroendocrine center are preserved.

Les glandes corpora allata manifestent une grande plasticité morphofonctionnelle dans divers états physiologiques et par rapport aux étapes du cycle biologique des insectes. Les modifications du volume sont dues à l'âge, à la nourriture, à la période vitellogénétique de l'ovaire ainsi qu'aux conditions expérimentales (allatectomie unilatérale, sectionnement des nerfs cardiaques, gonadectomie) [1] [5] — [7].

Certains auteurs attribuent l'augmentation du volume de la glande à la multiplication des cellules glandulaires ou à leur hypertrophie [4] [7]. L'aspect des cellules glandulaires diffère au cours du développement de certains insectes (*Oncopeltus fasciatus*) ; sombre chez les larves et claire chez adulte [2]. Il y a aussi des cellules polyploïdes, très riches en ADN chez *Chironomus* [4].

Le rôle des corpora allata en tant que coordinateurs du développement de la gamétogenèse, dirigeants de la capacité prolifique de l'insecte et conservateurs du degré de différenciation des tissus est bien connu. Les corpora allata manifestent ce rôle complexe en rapport étroit avec le cerveau et les corpora cardiaca [3] [9].

En tenant compte de l'étroite correlation morphofonctionnelle cerveau — complexe rétrocérébral, nous l'avons maintenu dans des cultures *in vitro* en y ajoutant de la gonade femelle, dans le but de poursuivre la réaction des cellules germinatives dans ces conditions.

MATÉRIEL ET MÉTHODE

On a cultivé *in vitro* dans l'hémolymphe du même individu le cerveau associé au complexe rétrocérébral et la gonade, prélevés chez *Gryllotalpa* femelle adulte (mois de juillet). Le milieu de culture utilisé a été dilué avec de la solution Ringer dans une proportion de 1:3. A ce milieu on a ajouté de l'ampicilline. On a suivi la réaction du tissu glandulaire

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des corpora allata, ainsi que celle des ovocytes après de courts délais de maintien in vitro (24 et 48 heures). Les corpora allata et les ovocytes ont été traitées ensuite par les techniques histologiques courantes.

RÉSULTATS

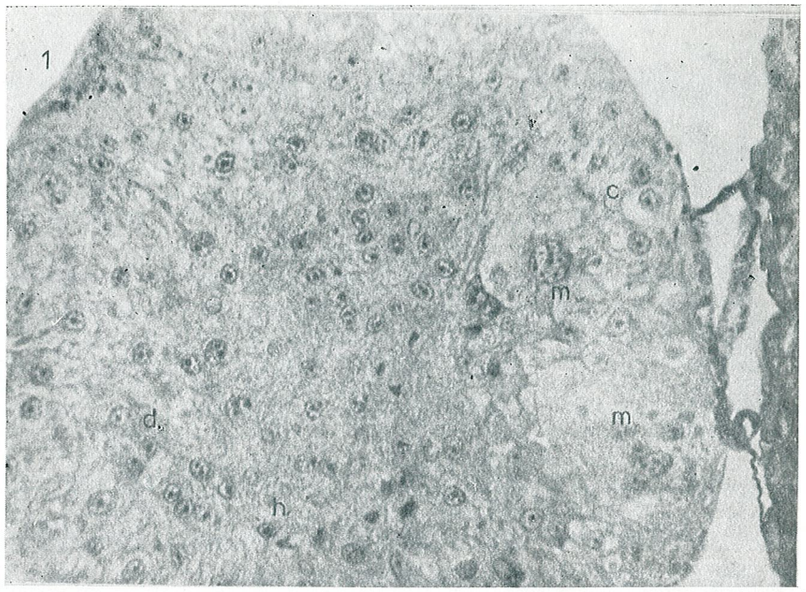
A la périphérie des corpora allata des femelles témoins on remarque une fine paroi conjonctive, qui envoie des replis plus ou moins profonds vers l'intérieur de la glande. Ces replis entourent les espaces étroits et de petites lacunes (fig. 1).

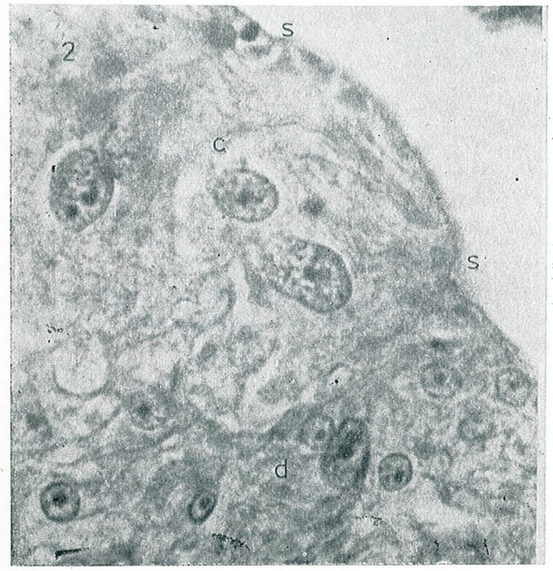
Les corpora allata sont pénétrés aussi par des fibres du nerf allate dont le trajet peut-être facilement repéré en suivant la position des noyaux des glyocytes périaxonaux parmi les cellulles glandulaires. Les cellules glandulaires périphériques, plus claires et plus grandes que celles centrales, présentent une polarisation évidente par rapport à la paroi et à ses replis conjonctifs. Au niveau du cytoplasme qui contient le noyau, la cellule est globulaire tandis que vers le paroi elle s'amincit. Les cellules plus profondes sont polyédriques plus foncés ou sombres et plus petites que les cellules périphériques. On trouve parmi les cellules glandulaires quellques-unes presque hypertrophiques et d'autres tout à fait contractées avec des noyaux picnotiques.

Les noyaux des cellules glandulaires ont une forme variable : sphérique, courbe, ovoïde et polymorphe. Dans le cytoplasme les grains de sécrétion sont isolés ou conflués en plages ; souvent ils sont amassés dans la région amincie des cellules claires. Parmi les cellules glandulaires on rencontre aussi des vastes territoires cytoplasmiques occupés par un seul noyau hypertrophique, sphérique ou polymorphe, qui dépasse de 10 à 12 fois la taille d'autres noyaux. Les nucléoles des noyaux hypertrophiques sont plus grands et plus nombreux. Souvent, le territoire cytoplasmique contient quelques noyaux isolés les uns des autres. Ceci peut nous conduire à considérer les masses cytoplasmiques multinucléés comme des formations plasmodiales. Les cellules qui entourent ces formations laissent l'impression d'avoir des parois incomplètes. Si ces cellules sont sur le point de se désintégrer alors leurs noyaux libérés peuvent pénétrer dans la masse cytoplasmique multinucléée qui représente dans ce cas un syncitium.

Les formations syncitiales ou plasmodiales peuvent peut-être régénérer de nouvelles cellules glandulaires. Nous avons constaté dans les corpora allata maintenus pendant 24 heures *in vitro*, l'existence de tous les aspects structuraux décrits chez le témoin, à l'exception de certaines cellules claires qui présentent des symptômes de dégénérescence par vacuolisation (fig. 3). Les vacuoles poussent le noyau vers la périphérie de la cellule ou forment, en confluant, un espace clair périnucléaire. Dans les deux cas, le noyau nous apparaît dégradé ; le nombre des nucléoles augumente et ils se blottent contre la membrane nucléaire. Sauf les cellules claires qui présentent des modifications visibles, les autres cellules glandulaires restent normales (fig. 4).

Après 48 heures de maintien *in vitro*, le nombre des cellules claires des corpora allata est bien réduit. Ces cellules, très volumineuses chez le témoin, se trouvent à l'étape de leur activité maximale. Leur dispari-





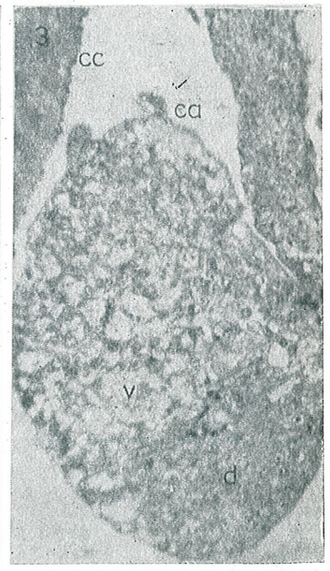


Fig. 1. — Corpora allata du témoin adulte. Cellule dense (*d*), cellule claire (*c*), cellule hyperchromatique (*h*), formation multinucléée (*m*).
Fig. 2. — Corpora allata du témoin adulte. Cellule claire (*c*) déversant sa sécrétion (*s*) à la surface de la glande.
Fig. 3. — Culture *in vitro* de 24 heures. Corpora allata (*ca*), corpora cardiaca (*cc*), vacuolisation des cellules claires (*v*), cellule dense (*d*).

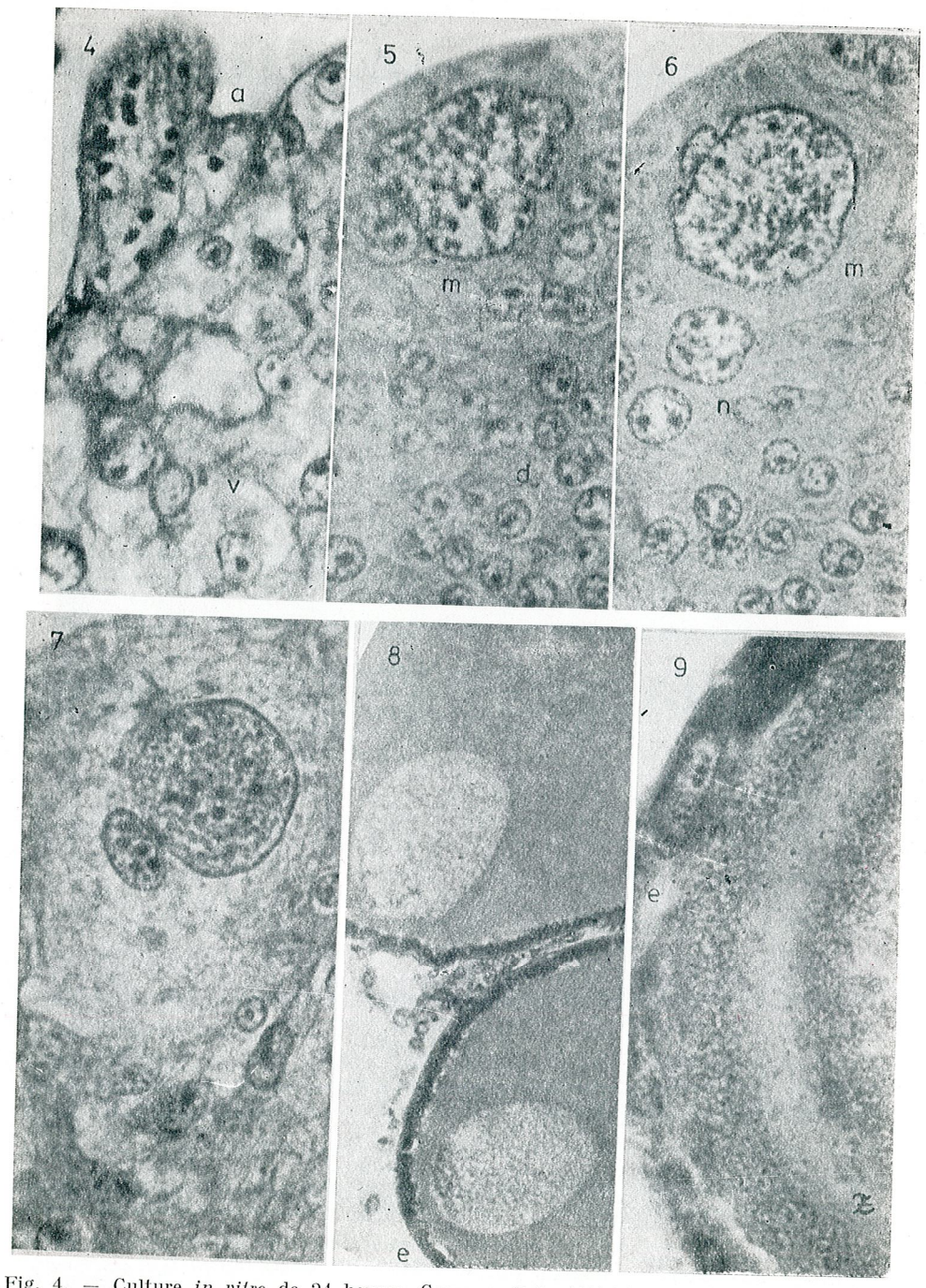


Fig. 4. — Culture *in vitro* de 24 heures. Corpora allata (détail) ; cellules claires en vacuolisation (*v*), noyaux pycnotiques dans les gliocytes du nerf allate (*a*).
Fig. 5, 6, 7. — Corpora allata après 48 heures de culture *in vitro* : les cellules denses (*d*) plus dominantes ; le bourgeonnement du noyau dans une formation multinucléée (*m*) ; noyaux des cellules hypertrophiques (*n*).
Fig. 8. — Ovocyte après 24 heures de culture.
Fig. 9. — Ovocyte après 48 heures ; zone réfringente (*z*).

tion 48 heures après l'expérience coïncide avec la diminution visible du volume de la glande. A leur tour, les cellules sombres diminuent aussi, leur cytoplasme devient une fine pellicule et le diamètre de leur noyau se réduit à moitié par rapport à celui normal.

On a pu constater que les cellules claires sont les plus sensibles, les premières à souffrir des altérations morphofonctionnelles *in vitro*. A la fin, après la modification des cellules centrales sombres, l'activité sécrétoire globale de l'organe diminue. Malgré le maintien des rapports anatomiques normaux avec le cerveau et les corpora cardiaca, les corpora allata gardent leur activité spéciphique seulement un court délai (environ 24 heures) pendant lequel le contrôle neuroendocrinien reste le même *in vitro* et *in vivo*.

Les recherches de Strong (1965) montrent que les glandes corpora allata actives ou inactives n'influencent pas le système génital quand elles sont implantées sans rapport avec le cerveau et les corpora cardiaca. Nos expériences gardent ce rapport. Ainsi les modifications ont eu la même intensité dans les gonades que dans les corpora allata.

24 heures après l'expérience, les ovocytes à n'importe quelle étape évolutive, ont l'épithélium folliculaire normal, ce qui constitue la preuve évidente que le milieu de culture n'est pas encore altéré (fig. 8). Tout comme chez le témoin, l'épithélium folliculaire des ovocytes jeunes et mûrs reste aplati, tandis que celui des ovocytes d'un stade intermédiaire est plié et contient des cellules sombres et cubiques.

48 heures après l'expérience, à la suite de la diminution de l'activité des corpora allata, surgissent des modifications nettes dans les ovocytes, aussi bien que dans l'épithélium folliculaire. On constate une déformation des ovocytes, accompagnée d'un changement de forme du noyau. A la périférie des ovocytes le cytoplasme est plus dense et plus réfringente que dans le reste, où l'on trouve concentrés les granules vitellins. Le territoire réfringent peut-être situé quelque fois autour du noyau, ou à une certaine distance de lui.

Pour une même ovocyte l'épithélium folliculaire subit des altérations régionales et devient par endroit soit aplati, soit cubique, soit haut et volumineux. Les cellules aplaties ont des noyaux fortement pycnotiques ; les cellules folliculaires les plus volumineuses acquièrent des fonctions phagocytaires et dans leur cytoplasme on remarquera des fragments de l'ovocyte altéré. Les troubles de la structure de l'ovocyte peuvent être imputée à la dégradation de l'épithélium folliculaire, le premier à enregistrer les éventuels changements chimiques du milieu de culture, à savoir la diminution de la quantité des hormones de la glande allata dans l'hémolymphe. Nos observations déja publiées ont montré la sensibilité de l'épithélium folliculaire des ovocytes et les phénomènes secondaires qui s'installent dans les ovocytes à la suite de la dégradation de cet épithélium sous l'influence des insecticides [10].

Discussions

Dorn avait signalé l'existence des cellules claires et denses dans le corps allate d'*Oncopeltus* ; les premières chez l'adulte est les autres seulement à l'état larvaire.

Chez *Gryllotalpa*, dans les corpora allata de l'adulte, nous avons trouvé des cellules claires ainsi que des cellules denses. De plus, on y trouve encore des cellules hypertrophiques, signalées aussi par Kiknadze et collab. chez *Chironomus*.

Ces cellules, ainsi que les formations multinucléées décrites par nous, caractérisent l'étape d'activité maximale de l'organe. Pendant nos expériences, quand le nombre des cellules claires diminue, on remarque aussi la réduction du volume de la glande allate, l'amoindrissement étant évident après 48 heures de conservation *in vitro*. Le cerveau des insectes influence directement l'activité de la gonade, mais ce stimulant est intensifié par le complexe rétrocérébral, surtout celui de corpora allata. Une preuve en est apportés par nos expériences qui montrent que le développement des ovocytes est affecté alors que dans le milieu de culture les corpora allata, le cerveau et l'ensemble du complexe rétrocérébral avec lesquels elles forment une unité morphofonctionnelle ne sont pas maintenus en rapports anatomiques. L'isolement d'un constituant nerveux ou endocrin de cette unité peut provoquer une dégradation dans tout les composants de l'unité, ainsi que des phénomènes compensatoires dans l'organe pair qui ont été déjà mentionnés par V. J. A. Novak (1966). Nous avons constaté de nouveau que les changements des concentrations des hormones neuroendocrines dans le milieu de culture sont perçus d'abord par l'épithélium folliculaire et ensuite par les ovocytes, phénomène qui prouve la sensibilité et la labilité morphofonctionnelle de cet épithélium [10] [11].

CONCLUSIONS

1. Les corpora allata de la *Gryllotalpa* adulte témoin comprend des cellules claires et denses, qui représentent peut-être deux phases fonctionnelles du même type de cellule. On y trouve encore des formations multinucléées — source génératrice de nouvelles cellules glandulaires — ainsi que des formations hypertrophiques qui influencent beaucoup l'augmentation du volume de la glande active.

2. La culture *in vitro* du corpora allata détermine : premièrement l'entrée en vacuolyse des cellules claires et puis leur disparition graduelle, qui correspond aussi à l'amoindrissement et à la dégradation de plusieurs cellules denses. A la suite, le volume et la sécrétion du corpora allata diminuent.

3. Dans la gonade, d'abord dans l'épithélium folliculaire et secondairement dans les ovocytes, s'installent des altérations qui correspondent à l'intensité des modifications surgies dans les corpora allata.

4. Les expériences prouvent aussi l'importance de la conservation de la glande corpora allata dans l'ensemble cerveau — complexe rétrocérébral, pour le déroulement de son activité spécifique et la coordination fonctionnelle de la gonade.

BIBLIOGRAPHIE

1. Ciudakova I. V., Bogarova-Messner O. M., Romaşkina M. P., 1975, Ontogenez, *6*, 20.
2. Dorn A., 1973, Z. Zellforsch., **145**, 447.
3. Girardie J., Girardie A., Moulins M., 1975, Gen. comp. Endocrin., **25**, 416.
4. Kiknadze I. I., Panova T. M., Novak V. J. A., 1976, Ontogenez, *6*, 11.
5. Lender T., Leverdue A. M., 1964, C. R. Acad. Sci., **258**, 1086.
6. Novak V. J. A., 1966, *Insect Hormones*, Ed. Methuen, London.
7. Scharrer B., 1964, Z. Zellforsch, **62**, 125.
8. Scharrer B., Harnack M., 1968, Biol. Bull., **121**, 193.
9. Strong L., 1965, J. Insect Physiol., **11**, 135.
10. Teodorescu M., Trandaburu V., Văcaru A., 1974, Rev. roum., Biol., **19**, 101.
11. Teodorescu M., Trandaburu V., 1976, Rev. roum. Biol., **21**, 61.

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OSTEOLOGICAL CHARACTERS OF THE SUBFAMILY *ACHEILOGNATHINAE* IN RELATION TO ITS PHYLETICAL POSITION *(PISCES, CYPRINIDAE)*

BY

CONSTANTINA SORESCU

The present paper deals with the study of the osteological characters within the subfamily *Acheilognathinae*, comparatively to other *Cyprinidae*, in order to clarify its phyletical relationships.
The author considers as phyletically significant only those osteological characters which remain constant within the subfamily and characterize it. The modifications of the skull bones are correlated with the evolution of the extensibility of the mouth, with the variations of the pectoral girdle, of the caudal fin, and of the Weberian apparatus. It is concluded that the subfamily *Acheilognathinae* is a primitive one, showing some similarities with the *Leuciscinae*, yet its osteological characters define it as an independent taxon.

The studies on the comparative osteology of the subfamilies *Leuciscinae*, *Abramidinae*, *Xenocypridinae*, *Danioninae*, *Cultrinae*, *Gobioninae*, *Barbinae* and *Cyprininae*, carried on in previous papers [3—7] on a great number of European, East- and South-Asian and North American species, living in various habitats, have demonstrated that not all osteological characters have a phyletical significance. Only those characters can be considered as phyletical significant, which remain constant within the subfamily and can thus be used in its diagnosis. Only the following bones of the skull are constant within each subfamily but differ from one subfamily to another : the supraethmoid, the frontals, dermopterotics, prevomer, parasphenoid and the interorbital septum. The evolution of the extensibility of the mouth is correlated with the shape of the supraethmoid. The bones of the pectoral girdle and of the Weberian apparatus have also phyletical value [5], but the osteological modifications of the caudal fin are the results of convergence [7].

The purpose of this paper is to try to give an osteological diagnosis of the subfamily *Acheilognathinae*, using the above-mentioned characters and to clarify, with their help, the relationships of the subfamily. Three species were used for the study : *Rhodeus sericeus amarus* from Europe ,*Paracheilognathus rhombeus* and *Acheilognathus cynostigma* from Japan.

The skull of the *Acheilognathinae* is characterized by : the supraethmoid with a slight concavity and reduced antero-lateral extensions ; prevomer with short and thick lateral processes ; parasphenoid narrow

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anteriorly, broad posteriorly; anterior margin of the frontals almost equal to the posterior one; interorbital septum high, with extended membraneous part, mouth slightly protractile (Figs 1 and 2).

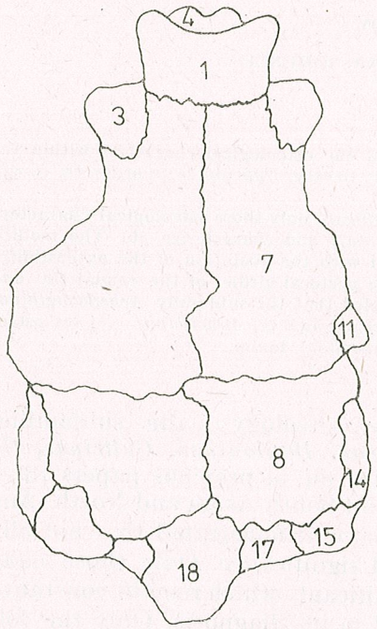

Fig. 1. — Neurocranium of *Rhodeus sericeus amarus* (subfam. *Acheilognathinae*) — dorsal view.

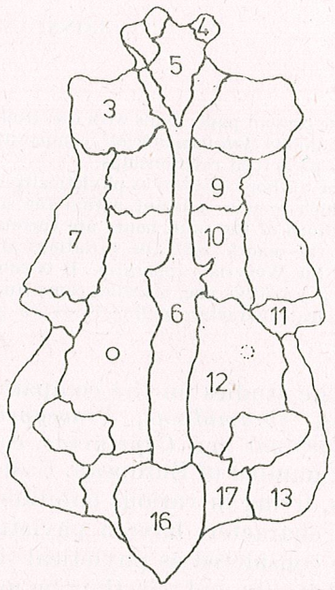

Fig. 2. — Neurocranium of *Rhodous sericeus amarus* — ventral view.

Figures common to all illustrations: *1*, supraethmoideum; *2*, mesethmoideum; *3*, ectethmoideum; *4*, preethmoideum; *5*, prevomer; *6*, parasphenoideum; *7*, frontal; *8*, parietal; *9*, orbitosphenoideum; *10*, pleurosphenoideum; *11*, sphenoticum; *12*, preoticum; *13*, pteroticum; *14*, squamosum; *15*, epioticum; *16*, occipital basilar; *17*, occipital lateral; *18*, crista occipitalis; *19*, palatinium.

The Weberian apparatus is characterized by: ascendent process of the scaphium well developed and triangular; articular process with a single ramification; within the composed vertebra, v_1 is plane-concave, v_2 amphicoelic and fused with v_3; ossa suspensoria of v_4 strong and with a vertical position.

Through the characters of the skull, of the pectoral girdle and of the Weberian apparatus, the *Acheilognathinae* is a primitive subfamily of *Cyprinidae*. Its osteological characters recall those of the *Leuciscinae* (Figs. 3 and 4), yet define it as an independent taxon.

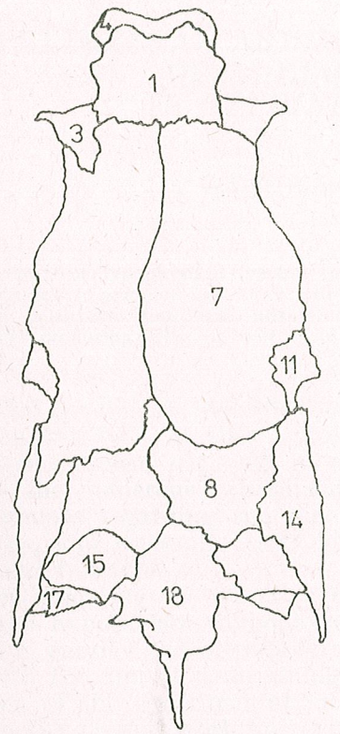

Fig. 3. — Neurocranium of *Aspius aspius* (subfam. *Leuciscinae*) — dorsal view.

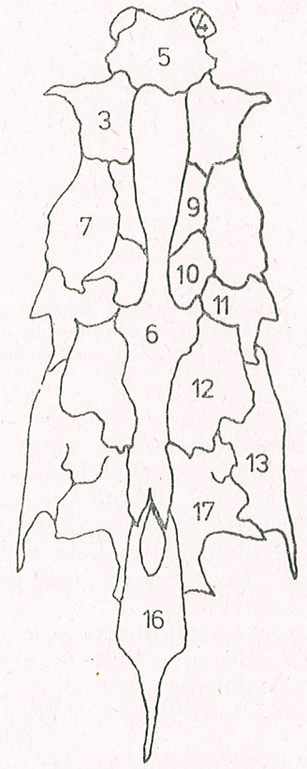

Fig. 4. — Neurocranium of *Aspius aspius* — ventral view.

REFERENCES

1. Gregory W., 1933, Trans. Amer. Phil. Soc., **22**, 1—48.
2. Sagemehl M., 1891, Morph. Jahrb., **17**, 489—595.
3. Sorescu C., 1969, Analele Univ. Craiova, **11**, 495—505.
4. — 1970, Rev. roum. Biol., Zool., **15**, *4*, 239—245.
5. — 1971, St. şi cerc. zool., **23**, *4*, 319—329.
6. — 1975, Rev. roum. Biol., Zool., **20**, *4*, 237—240.
7. — 1976, Studii şi cerc., Subcom. Ocrot. Monum. Nat., Slatina, 274—280.

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EXPERIMENTAL AND ULTRASTRUCTURAL STUDY OF ADENOHYPOPHYSIS IN *CYPRINUS CARPIO* L.

BY

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In the adenohypophysis of *Cyprinus carpio* L. the authors describe two cell-types with protein granules and three cell-types with glycoprotein granules. Following colchycine treatment ultrastructural changes have been observed in the STH producing cells, as well as in FSH producing ones.

The functional identification of various adenohypophyseal cell-types on the basis of their cytological characters is an incompletely elucidated problem not only in mammals, but also in low vertebrates. Despite the numerous experimental investigations there are contradictory opinions regarding the functional role of some adenohypophyseal cell-types, and especially their localisation and hypophyseal hormone-producing function.

Investigating the effect of cytotoxic and tranquilizing compounds upon the hypothalamo-hypophyseal system in the carp [13], we have studied in parallel the ultrastructural aspects of the adenohypophyseal cells with the aim of determining their possible modifications as a consequence of the experimental interventions.

In the present work our experimental results concerning the ultrastructural modifications of adenohypophyseal cells in carp will be shown under normal and experimental conditions.

MATERIALS AND METHODS

Specimens of carp (*Cyprinus carpio* L.) of 250—280 g were used for experiments. The animals from the experimental group were injected subdurally with a single dose of 1.6 mg colchycine/kg h. w. The animals were sacrificed by decapitation. The hypophysis was fixed in Bouin fixative for examination under a light microscope, and embedded in paraffine. For staining of the sections the following methods were used: AZAN according to Heidenheim, staining by Herlant and by Hurduc et al. For electron microscopy, the material was prefixed 90 minutes in 2.5% glutaraldehyde and thereafter was fixed in osmium tetroxide. For embedding Vestopal W was used. The sections contrasted with uranyl acetate and lead cytrate were examined by means of an electron microscope type TESLA BS-613.

RESULTS

The adenohypophysis of *Cyprinus carpio* is composed of three parts as follows: proadenohypophysis (PAH), i. e. pars distalis rostralis (PDR); mesoadenohypophysis (MAH), i. e. pars distalis proximalis

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(PDP); and metaadenohypophysis (MTAH), i. e. pars intermedialis. Cytologically, the most evolved and varied part is MAH.

PAH is made of type I protein cellular stripes (acidophils) and of a small number of glycoprotein cells (basophils) which are dispersed between the massive stripes of type I protein cells.

MAH mainly contains glycoprotein (basophil) cells and a reduced number of type II protein cells. In MTAH the acidophil cells predominate.

1. *Type I protein cells*, considered as somatotropic (STH) producing cells, have oval or polyhedric form and a maximum diameter of 8 to 10 microns, their cytoplasm being stained in pale-red with azocarmine. The nucleus of a great size is spherical or ellipsoidal. Ultrastructurally, these cells are characterized by the presence of electrondense proteidic granules. The granules are spherical or elongated, their diameter varying between 180 and 420 millimicrons. They are mainly concentrated at the pole of the cells.

The type I protein cells contain a developed rough endoplasmic reticulum (RER). The flattened cisternae of RER are more evident around the nucleus, where they are placed parallelly with the nuclear membrane. The relatively slightly outlined elements of the Golgi complex are present in the peripheral region of the cells. The mitochondriae, of small dimensions, are often very elongated (Fig. 1).

In the colchycine-treated individuals, one may observe a marked dilatation both of cisternae of the RER, and of cisternae of the smooth endoplasmic reticulum (SER) (Fig. 2). As a consequence of the dilatation of these ultrastructural elements, the cytoplasm has a vacuolized aspect. The vacuolization of the cytoplasm is reflected by the occurrence of some large vacuoles, containing a reduced amount of electrodense substances.

2. *Type II protein cells*, considered as prolactin or prolactinoform producing cells, have small dimensions (with a diameter of 7—9 μ), they are oval and are located between the great glycoprotein cells of MAH. They have large nucleus and cytoplasm with spheric uniform electrondense granules having a diameter ranging between 150 and 200 mμ. RER is slightly developed; a great number of free ribosomes are found in the hyaloplasm. The Golgi complex is mainly concentrated in the perinuclear area. The mitochondriae are significantly large and elongated (Fig. 3).

In the treated animals we did not observe any obvious ultrastructural modification at the level of these cells.

3. *Type III protein cells* are represented by some large polyhedric cells which have prolongations and are located in MTAH. The diameter of these cells changes between 12 and 15 μ. These cells, forming the major mass of MTAH, appear in two forms: some of them have a clear low electrodense and qualitatively reduced cytoplasm, others having a dark and intensively electrondense abundant cytoplasm (Fig. 4.).

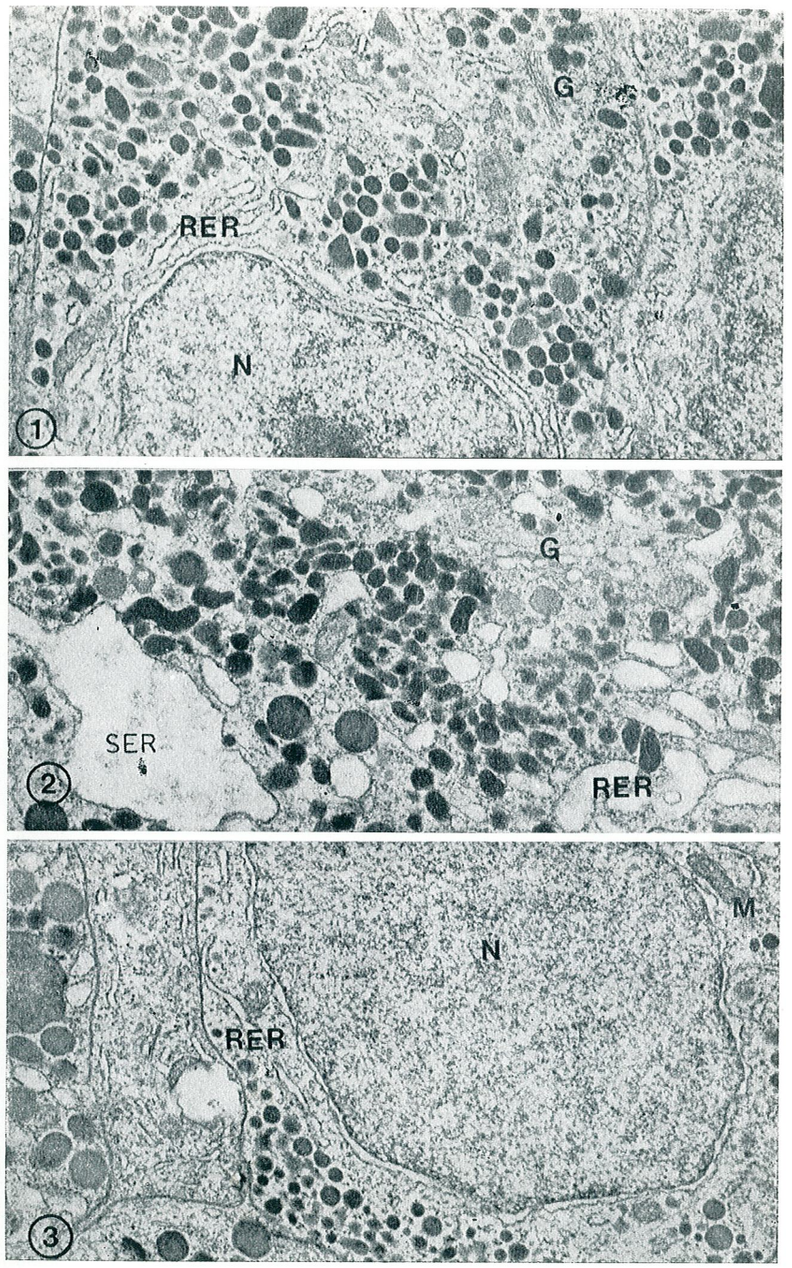
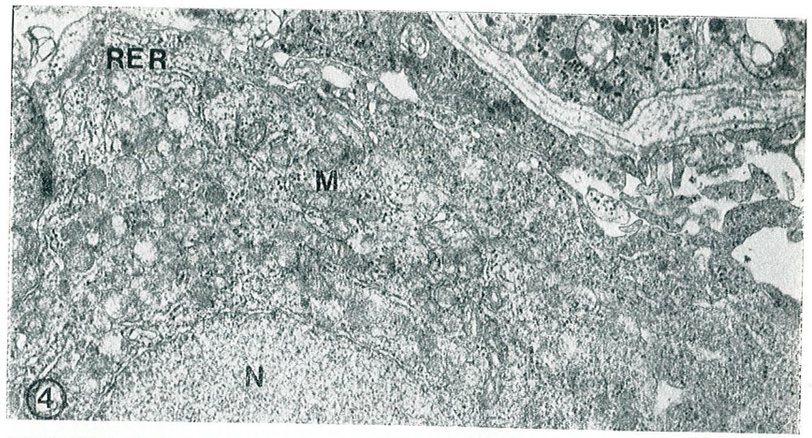
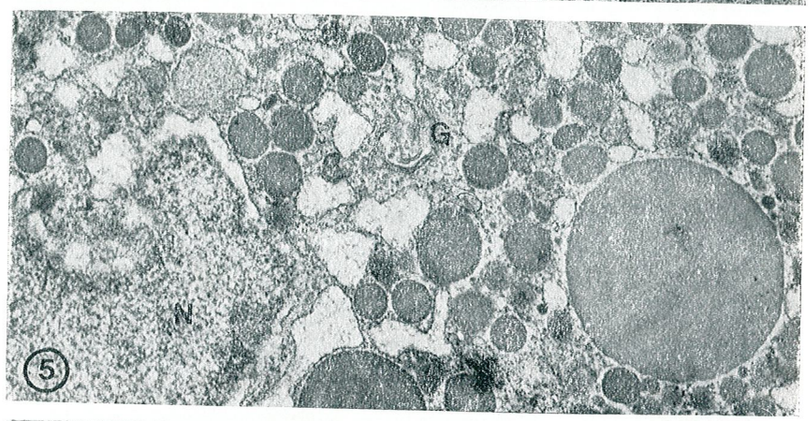


Fig. 1. — Type I protein cell in a control animal with granules of 180—420 mμ diameter, and with flattened cisternae of RER located parallelly with the nuclear membrane (X=17,000).

Fig. 2. — Type I protein cell in a colchycine-treated animal. The cisternae of RER are very dilated. (X=17,000).

Fig. 3. — Type II protein cell, with 150—200 mμ diameter granules (X=17,000).





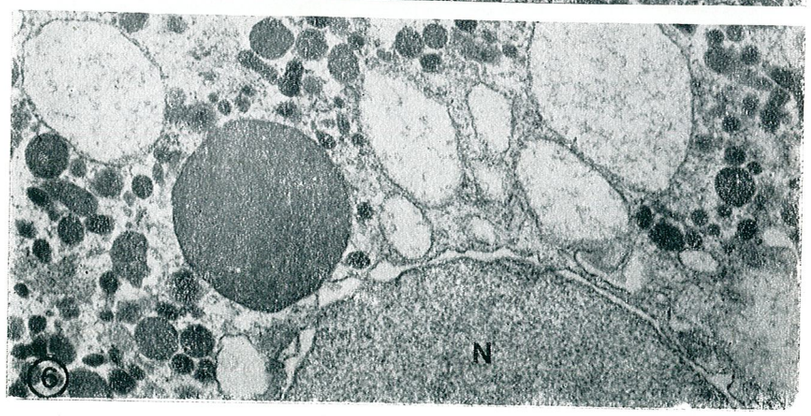


Fig. 4. — Type III cell (amphophil) with low electrondense granules and with well evolved RER (X=17,000).
Fig. 5. — Type I glycoprotein cell with granules diameter (between 400 and 500 mμ) and with globules of great dimension (2—3 μ diameter) (X=17,000).
Fig. 6. — Type I glycoprotein cell in a colchycine-treated animal. The cytoplasm is obviously vacuolized (X=17,000).

These cells with dark cytoplasm, named also zeta or ACTH producing cells of MSH, have a large spheric nucleus with a very well structured nucleolus. A well developed RER, with a great number of flattened sacs containing numerous polyribosomes, is present in the cytoplasm. There are also secretory vesicles throughout the cytoplasm and a large variety of electrondensity, changing between the very low and very evident electrondense forms.

4. *Type I glycoprotein cells* (basophil), are represented by two well distinct cell-types. Those in the first type have a great dimension, with a diameter of 14—20 μ, are located at the level of MAH and form cell-groups. Some of them are found in PAH.

The cytoplasm of these cells is abundant in sufficiently electrondense granules with a diameter ranging between 400 and 500 mμ. It also has 15—20 lower electrondense globules with a diameter reaching 2—3 μ (Fig. 5).

The SER contains numerous moderately dilated cisternae, a great number of little vesicles and some granule-containing vacuoles. The mitochondriae are little, the Golgi complex is not fully developed and is present in the perinuclear area. The relatively little nucleus is triangular (in section) and is often deformed.

In the colchycine-treated individuals especially a pronounced vacuolisation of the cytoplasm may be observed. The number of the vacuoles significantly increases, and the cisternae of REN become more dilated (Fig. 6). The granules are differentiated into two categories: some of them are little (approx. 200 mμ) and electrondense, while the others are large (450—500 mμ diameter) and low electrondense. The perinuclear space is more evident.

5. *Type II glycoprotein cells*, are represented by some little cells (with a diameter of 12—20 μ) which are situated in mesoadenohypophysis MAH. The cytoplasm has a reduced number of low electrondense granules, with a diameter of 180—300 mμ, and some large globules with a diameter of 1.5—2 μ. The RER is well developed, and mitochondriae are very elongated. The cisternae of the REN are greatly dilated.

In this type of cells we did not observe any evident ultrastructural modification following colchycine-treatment.

DISCUSSIONS

The adenohypophysis of the Teleosts was studied by a great number of researchers who investigated experimentally, biochemically and ultrastructurally the hypophyseal cell types. [6], [8], [4], [5], [10], [1—3], [9], [11], [10].

On the basis of the criteria of tinctorial affinity and ultrastructural characters the above-mentioned investigators have delimited

five or six cell-types. Although the tinctorial affinity and the ultrastructural analogies of hypophyseal cells in fishes do not prove sufficiently their endocrine functions as in mammals, it may be stated that the cytological delimitations of adenohypophyseal cells in fishes correspond to an adequate functional delimitation.

On the basis of cytological characteristics, we delimited five cell-types in the adenohypophysis of the carp : three cell-types with protein granules, and two types with glycoprotein granules. The type I protein cells — considered as STH producing ones — forms the major mass of PAH, and suffer obvious ultrastructural modifications following the action of colchycine. We did not observe similar changes at the level of the type II protein cells, named also prolactinoform hormone-producing cells.

The type II protein cells — ACTH and MSH producing cells — both in the controls and in the treated animals present very multiple aspects, but we were not able to demonstrate any evident ultrastructural modification.

Among the two types of glycoprotein granule-containing cells, ultrastructural changes (obvious vacuolization, exaggerated dilatation of the cisternae of RER, etc.) showed only the type I cells, considered as FSH producing ones.

From our investigations it follows that colchycine-induced ultrastructural modifications of different adenohypophyseal cell-types in carp are obvious on the one hand and insignificant on the other hand. This fact may be explained either by the selective effects of cholchycine upon the adenohypophyseal cells, or the characteristic specificity of these cells.

Taking into consideration our previous observations [13] concerning the blocking effect of colchycine upon the rapid transport of preopticohypophyseal neurosecretory product, we assume that colchycine acts upon the adenohypophyseal cell through the preoptico-hypophyseal tract as well as through the system of neurovascular connections.

CONCLUSIONS

By means of an electron microscope, on the basis of ultrastructural characteristics, in the adenohypophysis of carp (*Cyprinus carpio L.*) we distinguished five types of cells : three types with protein granules, and two types with glycoprotein granules. Following colchycine treatment of the animals, we noticed obvious ultrastructural modification in type I protein cells (STH producing cells), as well as in the type I glycoprotein cells (FSH producing cells). We assume that the effect of colchycine is realized through the preoptico-hypophyseal tractus as well as through the neuro-vascular connection systems of the hypophysis.

REFERENCES

1. Follénius E., 1963, Arch. Anat. micr. Morphol. exp., **52**, 429.
2. Follénius E., 1964, C. R. Soc. Biol. (Paris), **158**, 1942.
3. Follénius E., 1968, Path. Biol., **16**, 619.
4. Mattheij J. A. M., 1968, Z. Zellforsch, **90**, 542.
5. Mattheij J. A. M., 1969, Z. Zellforsch, **101**, 588.
6. Olivereau M., 1963, Gen. Comp. Endocrinol., **3**, 312.
7. Olivereau M., 1965, Gen. Comp. Endocrinol., **5**, 109.
8. Olivereau M., 1968, Z. Zellforsch., **87**, 545.
9. Öztan N., 1966, Z. Zellforsch., **69**, 699.
10. Pickford G. E., Atz J. W., 1957, *The physiology of the pituitary gland of fisches*, New York, New York Zool. Soc.
11. Szabó Zs., Molnár B., 1968 Rev. roum. Endocrinol., **5**, 209.
12. Szabó S., Molnár B., 1969, St. cerc. Biol. ser. Zool., **21**, 321.
13. Szabó S., Crăciun C., Molnár B., 1976, Rev. roum. Morphol. Embryol. Physiol. sér. Morphol. Embryol., **12**, 157.

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INVOLVEMENT OF CAROTENOIDS IN EMBRYOGENESIS OF *SALMO GAIRDNERI*

BY

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K. BATHES, R. BRANDCH

Spawn of *Salmo gairdneri* incubated for 24 h was maintained either in running water or in an anoxic medium, for another 24 hours.
In spite of the very low cytochrome oxidase activity registered in anoxic eggs, the pattern of carotene metabolism was identical with that found in eggs developing in normal conditions.
Our results point out that the carotene substances play a part in a functional energy transfer system which parallels the cytochrome system.

INTRODUCTION

It is generally accepted [1] [4—5] that the early stage of embryogenesis is an anoxybiotic process. In 1960, Brachet [3] proposed that there might be some "additional" metabolic pathways different from the oxidative ones, which would explain the behaviour of fish eggs in anaerobiosis.

Works carried out on Salmonide eggs [16] tried to clarify the problem of resources and the sequence of their utilization during the embryonic development. However, Brachet's problem still remains open. Since our previous results [8] showed the effective participation of carotenoids in embryogenesis, in the present paper we extended our investigations to see whether these pigments may serve as "additional" pathway for the general embryogenetic metabolism.

MATERIAL AND METHODS

In one experiment, *Salmo Gairdneri* spawn, were divided into three equal lots 24 h after fecundation. In the control group the carotenoids were immediately extracted, whereas the eggs of the second group were maintained for another 24 h in running water at room temperature and in darkness. The third lot kept in the same conditions of humidity, darkness and temperature, was maintained in water with low oxygen content (1.95 mg O_2/1) [17] in a tightly closed vessel, the oxygen being removed by bubbling the water with nitrogen. A second experiment, under exactly the same conditions, was performed on eggs 48 h after fecundation. In both experiments the relative anoxia achieved lasted for 24 hours.

The total carotenoids extracted in acetone-ethyl ether (1 :3) were processed as previously reported [8]. Chromatography on Silica gel-Celit (3 :1) (Silicagel Merck, Celit BF Serva) isolated the main carotenoid fractions which were to serve to their own quantitative identification to the determination of lipid-carotenoid complex in each fraction [18] as well as for separating different pigmentary forms from each fraction.

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The pigment purification by TLC [7] made possible the recording of IR spectra of control samples using a "Specord 71 IR" and a Perkin-Elmer apparatus model 337 for the spectra of experimental samples, as a capillary layer between KBr windows.

The identification of pigments was made as previously described [8]. The stage of embryonic development was histologically established and the respiratory energy was evaluated from cytochrome oxidase activity [11]. The data were statistically processed using the Student "*t*" test.

RESULTS AND DISCUSSION

After 24 hours of incubation in a normal or a relative anoxic medium, there was no difference in the segmentation of eggs maintained in these two media. Even spawn 48 h after fecundation placed in a poor oxygen medium developed blastulas identical with those from the control group.

Our observations are in agreement with the data reported by Devillers and Rosenberg [4] who kept eggs of *Salmo irideus* in cooled boiled water for 48 h immediately after fecundation and observed a bipolar differentiation and a normal segmentation. Then, if the anoxia were prolonged, a progressive obstruction of differentiation occurred, resulting in death at gastrula stage.

The analysis of egg cytochrome oxidase activity revealed a sharp increase immediatly after fertilization lasting for a further 24 h if the eggs were maintained in running water. Contrariwise, a significant inhibition of the enzyme activity was observed in eggs kept 24 h in poor oxygen medium compared to the normal group (Table 1).

Table 1

The cytochromoxidasic activity in µl O_2/h/g wet tissue

Control	Eggs incubated 24h+24h maintained in running water	Eggs incubated 24h+24h in an anoxic medium
$\bar{x}=45.35$	$\bar{x}=135.18$ $t=7.36$ $p>0.001$ (6	$\bar{x}=64.88$ $t=4.94$ $p>0.001$

Column chromatography of total extracts of pigments from control eggs and those incubated for 24 h in the two media, revealed the same number of fractions. After purification of each fraction by TLC, several pigmentary forms were separated and identified on the basis of their partition coefficient and the specific vibrational absorption bands in IR and visible spectra.

First, a band appeared between 3460 and 3360 cm^{-1} which attests the presence of alcohols. Then, there were vibration bands between 1045—1025 cm^{-1} and 1160—1153 cm^{-1}, corresponding to the absorption bands of secondary, respectively tertiary, OH groups vibrations [9] [15].

The characteristic band of certain esterified hydroxylic groups was present in spectrum between 1750—1730 cm^{-1}.

Considering the ratio of relative intensities of the characteristic bands for OH groups (3420—3380 cm^{-1}) and those for ester groups (1750—1730 cm^{-1}) as well as the ratio of relative intensities of *cis* (1360—1370 cm^{-1}) and *trans* (3000—3010 cm^{-1}) forms, expressed in mm, it is evident that the carotenoid alcohols present in fractions I and II are almost completely esterified after 24 h of normal embryogenesis, but only partially esterified when the development occurred in a hypoxic medium (Table 2).

Table 4

The ratio of the relative intensity of the vibration bands *cis-trans* (in mm)

Control	Fractions I and II	Fractions III
	2.66	3
Eggs incubated 24h+24h maintained in running water	0.55	0.86
Eggs incubated 24h+24h in an anoxic medium	0.60	1.0

Table 3

The quantity of lipids from each carotenoid fraction

Control	Fractions I and II	Fraction III
	2.582 ± 0.25 (6	1.154 ± 0.14
Eggs incubated 24h+24h maintained in running water	2.604 ± 0.22 $t=0.07$ (7	0.597 ± 0.055 $t=3.71$ $p<0.01$
Eggs incubated 24h+24h in an anoxic medium	2.362 ± 0.25 $t=0.63$ (6	0.373 ± 0.066 $t=5.04$ $p<0.001$

Table 2

The relation of the relative intensities of OH groups and the esteric groups (in mm)

Control	Fractions I and II	Fractions III
	0.80	0.18
Eggs incubated 24h+24h maintained in running water	0.07	0.20
Eggs incubated 24h+24h in an anoxic medium	0.10	0.32

Table 5

The quantity of carotenoid pigments from each fraction

Fractions I and II			Fraction III			Fraction IV		
Control	Eggs incubated 24h+24h maintained in running water	Eggs incubated 24h+24h in an anoxic medium	Test	Eggs incubated 24h+24h maintained in running water	Eggs incubated 24h+24h in an anoxic medium	Test	Eggs incubated 24h+24h maintained in running water	Eggs incubated 24h+24h in an anoxic medium
$\bar{x}=0.0389$	$\bar{x}=0.0512$	$\bar{x}=0.131$	$\bar{x}=0.329$	$\bar{x}=0.278$		$\bar{x}=0.133$	$\bar{x}=0.201$	
	$t=1.73$ $p<0.1$	$t=7.31$ $p<0.001$		$t=2.04$ $p<0.05$		$t=11.9$ $p<0.001$	$t=41.8$ $p<0.001$	
	→	$t=5.44$ $p>0.001$		→		→	$t=14.2$ $p<0.001$	

At the same time, the esterified alcohols from control eggs present in fraction III freed some of the hydroxylic groups, since the number of free hydroxyls increased in the experimental lots (Table 2). Their variations fit well with the total lipid content of the respective fractions (Table 3). Moreover, the ratio of relative intensities of *cis* and *trans* vibrational bands for the same pigments indicated a change in their spatial structure. Thus, the *cis-trans* form at the single band of the aliphatic chain, characterized by the vibrational bands at 1360 cm^{-1} and 3030 cm^{-1} changed after 24 h compared to the control (Table 4, Figs, 1—3).

Although present, the *cis-trans* vibrational bands at the double bond, revealed by the vibrational bands at about 720 cm^{-1} and 960 cm^{-1} respectively, do not seem to interfere with the embryogenetic process, since their position was not affected.

The transition from *cis* to *trans* configuration occurred with release of energy — probably high enough to support the labilization of carotenoid-lipid-protein complex. This is perhaps a process similar to the isomerization of "*cis*-11-retinaldehyde" in *trans* form, which primes the visual impulse [10].

Fraction IV was the richest in pigmentary forms, but the poorest in lipids. Apart from α-cryptoxanthin and lutein present in both control and incubated eggs, more esterified astaxanthin was found in anoxic spawn than in the other two groups.

The carotenoid forms contained in fraction III were partly used during the 24 hour incubation period (Table 5) whereas those from fraction I and II appeared in a higher concentration. These findings make us presume that these pigmentary forms either did not change, or an additional process of synthesis occurred (Table 5).

The purification of the pigments of these fractions by TLC together with the determination of partition coefficient revealed pigments with alcoholic character, one of which, with visible absorption maxima in light Petroleum at 470—440—418 mμ indicating the presence of Chloroxanthin.

Although in IR spectrum, absorption bands appeared at 1635 cm^{-1} and 977 cm^{-1} it is difficult to assume that they can be ascribed only to Chloroxanthin, since the esteric group also absorbs in range of 1835 cm^{-1} and the *trans* form in the range of 977 cm^{-1}.

However, the presence of chloroxanthin may plead for a special synthetic process taking place in anoxic eggs and would explain the quantitative increase of carotenoids in fraction I and II extracted from these eggs (Table 5). Whether this increase could be regarded as a compensation for the consumption of pigments from fraction III in spawn developing in a natural or an anoxic medium, it remains to be seen.

Our results clearly indicate that in relative anoxia the early .morphogenetic processes occurred normaly, in spite of the significant reduction of cell respiration through the cytochromic chain.

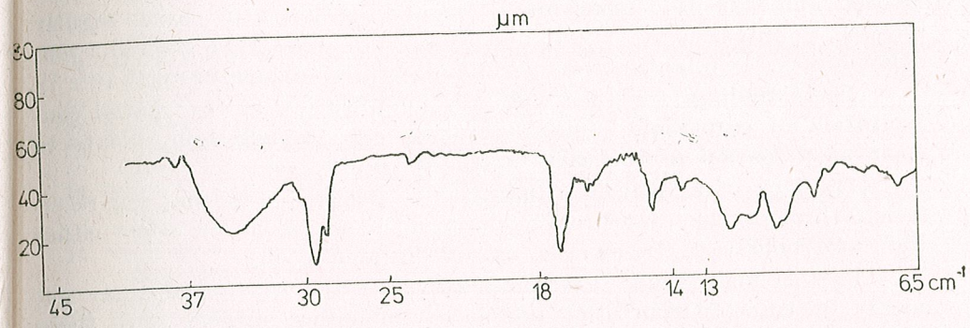

Fig. 1. — The recording of IR spectra. *Control*. The carotenoid form 3^1.

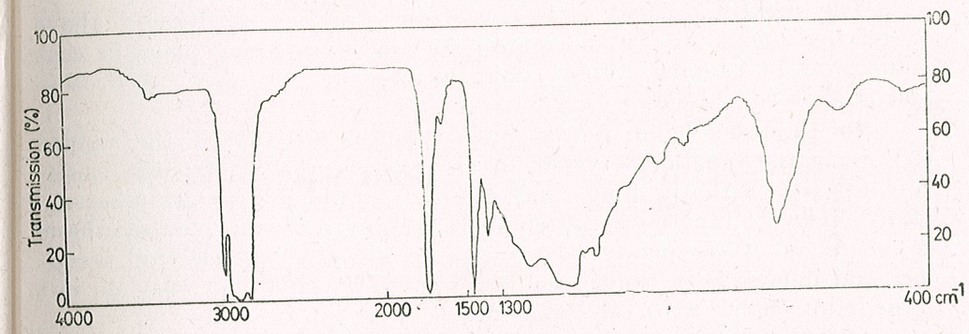

Fig. 2. — The recording of IR spectra. After 24 h incubation in running water. The carotenoid form 3^1.

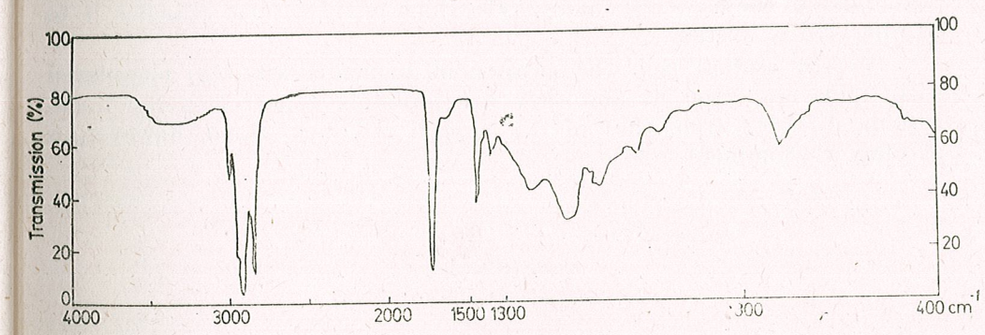

Fig. 3. — After 24 h incubation in hypoxic medium. The recording in IR spectra of the carotenoid form 3^1.

The process studied by us in spawn developing in natural or pseudo anoxic media had a similar course with the difference that the esterification and transition from *cis* to *trans* configuration were slightly reduced in intensity in pseudo-anoxic eggs. Instead, they showed a tendency to increase quantitatively certain of their carotenoid forms (Table 5).

These findings together with the modification in the spatial configuration of carotenoid pigments during egg segmentation, points to their participation in the early stage of embryogenesis.

The lack of a sufficient quantity of oxygen for normal egg respiration through the cytochromic chain does not seem to affect either the carotenoid activity, or the normal segmentation.

Preeble and Huda [12] concluded that the carotenoids may get energy by transfer from other molecules, including oxygen, when these molecules are in a "singlet" or "triplet" state of excitation, after returning to their normal state.

The slightly reduced intensity of the processes mentioned above in relative anoxic spawn in comparison to those taking place in eggs maintained in running water, could be explained in view of Preeble and Huda's conclusions.

On the other hand, it was already established that in the vegetal cell, along the functional system of chlorophyls, the carotenoids constitute together with the lipids and proteins to which they are noncovalently linked, "functional systems" playing a part in photosynthesis [6]. Although these pigmentary molecules are excellent electron acceptors and donors [13], Smith and Ramirez [14] claim that they do not form redox-chains with the cytochromes.

In Thiorodacea the pigmentary system includes carotenoids absorbing photons with higher energy than those absorbed by their chlorophyls. Consequently, it is the carotenoids that convey energy as "excitons" to the bacterial chlorophyls which in their turn, transfer it to another redox system.

In the animal cell, the carotenoid pigments are also complexed with lipids and proteins and they are probably a part of a functional system, different from that of the vegetal cell, but which, however, is playing a prominent role.

REFERENCES

1. Blaxter J., 1959, Fish Physiology, **V**, *III*, 203, Acad. Press, New York—London.
2. Bonaly R., 1957, *Biosynthèse des Caroténoides cycliques des levures du genre Rhodotorula*, Thésis, Nancy.
3. Brachet J., 1950, *Biochemistry of Development*, Pergamon Press, London.
4. Devillers Ch. Rosenberg J., 1955, C. R. Acad. Sci., **273**, 1561—62.
5. Devillers Ch., 1955, Ann. Biol., (F), *4*, *3—4*, 157—86.

6. Goodwin T. W., Mercer E., 1973, *Introduction to Plant Biochemistry*, 59 Pergamon Press, Oxford — New York.
7. Hager A., Meyer-Bertehenrath T., 1966, Planta (Berlin), **69**, 198—217.
8. Jitariu M., Chera E., Duca E., Link G., Rotinberg P., Szilagyi I., 1975, Rev. Roum. Biol. sér. biol. anim., **20**, *4*.
9. Liaaen-Jensen S., Jensen A., 1965, *Recent Progress in Carotenoid Chemistry*, Pergamon Press, London — New York.
10. Leslie R., 1958, *Biological Membranes*, Acad. Press, London — New York.
11. Pigareva Z., Cetverikov D., 1950, Biochimia, **15**, *6*, 517.
12. Preeble B., Huda A., 1973, Photochem. Photobiol., **17**, 255—64.
13. Pullman B. — Pullman A., 1963, *Quantum Biochemistry*, Interscience Publ., London.
14. Smith L., Ramirez J., 1964, J. Biol. Chem., **235**, *1*, 219.
15. Weedon B. C., 1965, *Chemistry-Biochemistry of Plant Pigments*, Acad. Press, London — New York, p. 80.
16. Williams J., 1967, *The Biochemistry of Animal Development*, Acad. Press, London — New York, **V**, *II*, 7.
17. Winkler J., 1972, *Protecția calității apelor*, Stas 6536—62, Ed. tehnică.
18. Woodman D., Price C., 1972, Clin. Chim. Acta., **38**, 39—43.

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LE POLYMORPHISME DES PROTÉINES AU COURS DU DÉVELOPPEMENT ONTOGÉNÉTIQUE CHEZ L'ESTURGEON (*ACIPENSER RUTHENUS* L.)

PAR

MARIA CALOIANU IORDĂCHEL et MARIANA SUCIU

The electrophoretic characteristics of proteins in different stages of ovary maturing in sturgeon, are described as well as the proteic polymorphism of hepatic and blood proteins as compared to the sexuate maturation. The electrophoretic characteristics of hepatic and blood proteins in the hybrid *Acipenser ruthenus* L. × *Acipenser stellatus* Pallas are also described.

Des résultats acquis jusqu'à présent avèrent l'existence de la spécificité du tableau électrophorétique et, par conséquent, la relation entre le polymorphisme des protéines et l'âge, le sexe et la maturation sexuelle, chez les poissons [1], [3], [5], [7], [8], [9], [10].

Le polymorphisme des protéines tissulaires en divers stades du développement ontogénétique relève l'évolution de la capacité des cellules pour une synthèse protéique orientée vers la synthèse d'un polypeptide spécifique à un moment donné dans la vie de la cellule. Les phénomènes sont traduits par l'apparition des nouvelles fractions protéiques, ou la disparition de quelques fractions existantes dans les stades antérieurs.

Par conséquent, l'étude des caractéristiques électrophorétiques permet de lier l'explication des phénomènes biochimiques aux événements de la différenciation morphologique.

Le présent travail décrit les caractéristiques électrophorétiques des protéines des gonades, du foie et des protéines sanguines chez l'esturgeon pendant le développement ontogénétique.

MATÉRIEL ET MÉTHODE

Les exemplaires d'*Acipenser ruthenus* L. ont été pêchés au chenal du Danube (le bras Borcea, la zone Feteşti). On a analysé des exemplaires longs de 22,5 cm—57,5 cm, au poids entre 50—700 g. La chance d'avoir pêché un hybride *Acipenser ruthenus* L. × *Acipenser stellatus* Pallas nous a permis d'effectuer des comparaisons supplémentaires.

Pour déterminer les modifications structurales des gonades on a utilisé les techniques classiques de fixation et d'analyse histologique ainsi que les méthodes habituelles d'étude électronomicroscopique.

Les analyses biochimiques ont été effectuées de la même manière que sur le foie et le sang des femelles et des mâles d'*Acipenser ruthenus* et du mâle hybride. On a effectué l'extraction des protéines et l'électrophorèse sur gel de polyacrilamide dans les conditions décrites dans une étude antérieure [10].

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RÉSULTATS

L'étude de propriétés électrophorétiques des protéines solubles a relevé des traits caractéristiques de cette espèce de poissons.

A. Ovaire. Au premier stade de la prévitellogenèse (stade II, correspondant à l'échelle de la maturation des gonades) les protéines des oocytes sont représentées par 6 fractions électrophorétiques (fig. 1 *A*). Chez les jeunes exemplaires avec des gonades dans une phase plus avancée

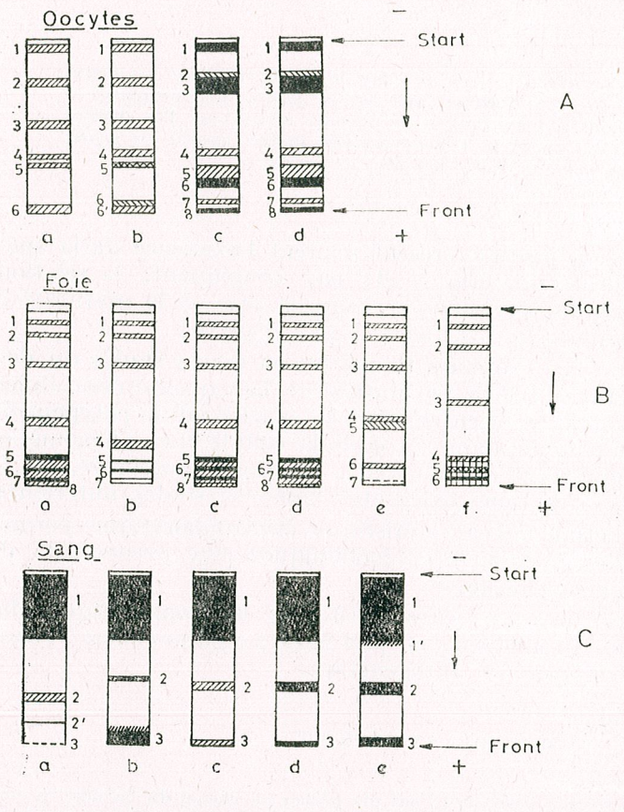


Fig. 1.— *Electrophorégrammes.*

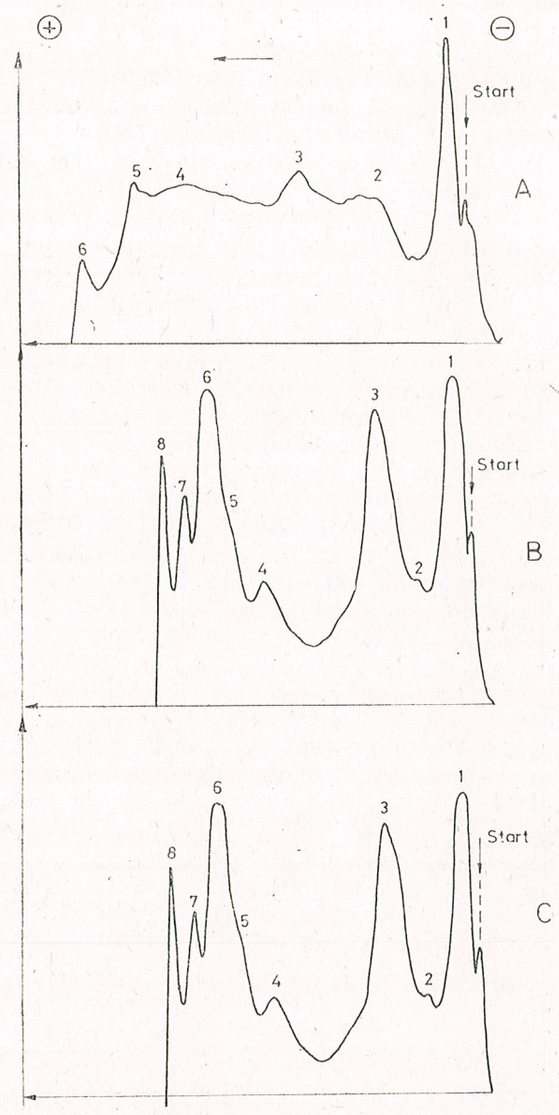
A, Le polymorphisme protéique chez les oocytes d'esturgeon au cours du développement ontogénétique; a) oocytes au stade II; b) oocytes au stade «II gras»; c) oocytes au stade III—IV; d) oocytes au stade final de développement. *B*, Le polymorphisme de protéines hépatiques; a) foie mâle adulte d'esturgeon; b) foie de jeune mâle d'esturgeon; c) foie de femelle adulte d'esturgeon; d) foie gras de femelle adulte d'esturgeon; e) foie de jeune femelle d'esturgeon; f) foie de mâle adulte (hybride *Acipenser ruthenus* × *Acipenser stellatus*. *C*, Le polymorphisme des protéines sanguines : a) sang de jeune mâle d'esturgeon b) sang de mâle adulte d'esturgeon c) sang de jeune femelle d'esturgeon d) sang defemelle adulte d'esturgeon e) sang de mâle hybride adulte

de la prévitellogenèse («stade II +», ou «stade II gras» —nommé ainsi à cause de la grande quantité de graisse accumulée au niveau de la gonade) on a constaté qu'à la place de la 6e fraction du stade antérieur, il y avait deux fractions (N° 6, et N° 6′) présentant une mobilité électrophorétique rapprochée. Le commencement du processus de la vitellogenèse (stade III de maturation) se traduit par l'apparition des inclusions deutoplasmiques dans le cytoplasme des oocytes.

Au cours du processus de la vitellogenèse (stade III — IV et stade IV) le tableau électrophorétique des protéines marque l'existence des modifications quantitatives et qualitatives des fractions. Dans ces stades

Fig. 2. — *Densitogrammes.*

A, oocytes stade II; *B*, oocytes stade III—IV; *C*, oocytes stade IV.



on rencontre 7 fractions protéiques. On peut remarquer la disparition de la 3e fraction du deuxième stade, ainsi que la prédominance du point de vue quantitatif de la 2e fraction.

Le fait que ces deux stades (III—IV et IV) présentent des caractéristiques électrophorétiques ressemblantes prouve la continuité du même type de synthèse protéique.

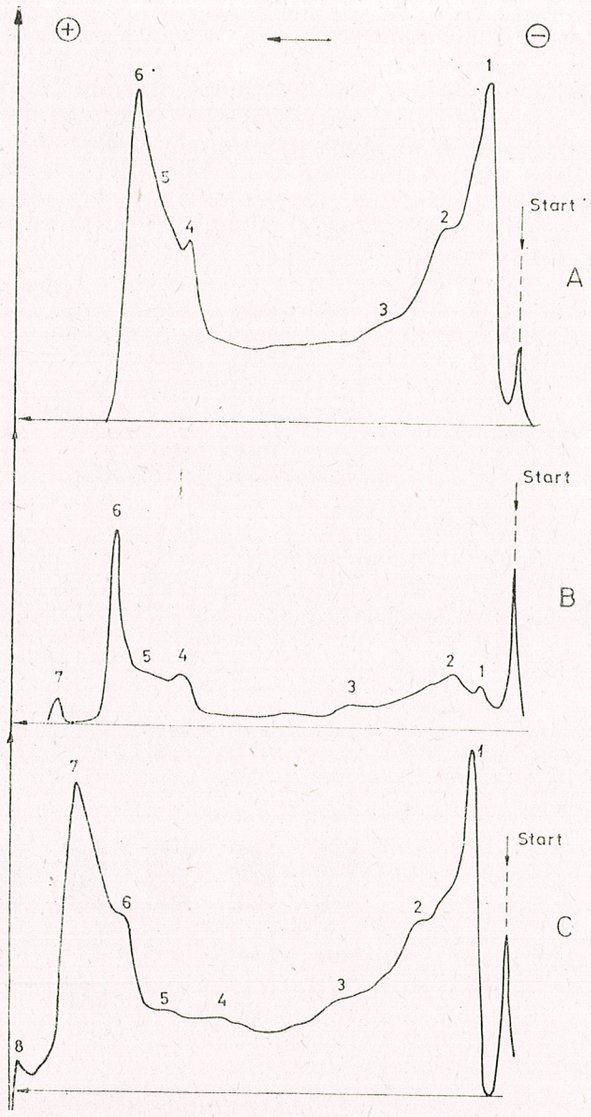


Fig. 3. — *Densitogrammes*. *A*, Foie de mâle hybride; *B*, foie d'un jeune esturgeon; *C*, foie d'esturgeon mâle adulte.

B. **Le foie** (fig. 1 *B*). Les tableaux électrophorétiques des protéines hépatiques montrent un polymorphisme évident par rapport à l'âge et au sexe des exemplaires étudiés.

Chez les femelles jeunes (stade II) on trouve 7 fractions dispersées uniformément dans le tableau électrophorétique; chez les femelles adultes (stade IV) on trouve 8 fractions de protéines hépatiques, mais la disposition est bien différente par comparaison au stade juvénile. C'est-à-dire au lieu des fractions notées avec 4—5, au stade II, il y a maintenant

une seule bande, tandis que les fractions 5—8 ont un caractère prononcé électropositif et sont groupées au front.

Chez les jeunes mâles on a constaté l'existence des 7 fractions protéiques; leur disposition est différente par comparaison aux jeunes femelles. Tandis que chez les femelles les bandes étaient disposées uniformément, les fractions 4—5—6—7 des protéines hépatiques des jeunes mâles sont groupées au front.

Chez les mâles adultes on constate l'existence d'une fraction nouvelle inexistante au stade juvénile, notée ici avec le N° 4.

C. **Le sang** (fig. 1 C). Selon l'analyse électrophorétique effectuée sur le sang des femelles et des mâles d'*Acipenser ruthenus* jeunes et adultes, on a constaté de nettes différences par rapport à l'âge et au sexe des individus.

Il y a une fraction intensément colorée, qui reste près du départ et qui est invariable chez tous les exemplaires étudiés. Dans le reste du tableau électrophorétique on a constaté des variations spécifiques. C'est-à-dire, chez les femelles jeunes il y a 2 fractions, hormis la première fraction invariable, lesquelles sont réduites du point de vue quantitatif. Dans le sang des femelles adultes on a constaté une augmentation quantitative de la fraction à mobilité électrophorétique moyenne.

En ce qui concerne les mâles, on a constaté que chez les jeunes il y a une fraction de plus, en comparaison des femelles du même âge. C'est-à-dire, la fraction N° 2′ — à mobilité électrophorétique moyenne. Chez les mâles adultes (stade IV) on a constaté la concentration de la fraction rapide N° 3.

D. **Le hybride** *Acipenser ruthenus* L× *Acipenser stellatus* (exemplaire mâle avec des gonades au stade III—IV de maturation). En ce qui concerne les protéines hépatiques, les électrophorégrammes obtenus (fig. 1 B) démontrent qu'il y a de nettes différences entre celui-ci et *Acipenser ruthenus* L.

Le tableau électrophorétique contient 6 bandes dont 3 fractions sont groupées au front. En ce qui concerne le tableau électrophorétique des protéines sanguines, il diffère de ceux obtenus des exemplaires du même âge d'*Acipenser ruthenus*. Il s'agit de la concentration de la fraction N° 2 et N° 3 et de l'existence d'une fraction nouvelle, la fraction N° 1′.

DISCUSSIONS

Le polymorphisme des protéines tissulaires est décrit dans la littérature comme un caractère spécifique. McAllister [8] affirme que les hybrides ont des fractions protéiques caractéristiques pour les tableaux électrophorétiques des deux parents. Johnson [7] signale la spécificité des protéines sarcoplasmiques chez une espèce de poissons mais, en ce qui concerne cette catégorie des protéines, il n'a remarqué aucun rapport avec l'âge ou le sexe.

Pech [9] a étudié les caractéristiques électrophorétiques des protéines sanguines chez les poissons et il a constaté la concentration spéci-

fique des fractions au moment de la maturation des gonades et l'existence d'une fraction spécifique pour les mâles, et une pour les femelles.

Les données obtenues pour *Acipenser ruthenus* L. nous permettent d'affirmer qu'en ce qui concerne les oocytes il s'agit d'un polymorphisme des protéines solubles au cours de la maturation de la gonade.

An niveau des protéines hépatiques nous avons constaté l'existence d'un polymorphisme par rapport à l'âge et au sexe, c'est-à-dire des modifications évidentes au cours du développement ontogénétique, illustrées par l'apparition de nouvelles fractions chez les adultes et la disparition des fractions existantes dans les stades antérieurs.

Chez les individus adultes on n'a pas remarqué l'existence du polymorphisme sexuel en ce qui concerne les protéines solubles du foie. La comparaison effectuée à l'aide de l'exemplaire hybride a confirmé l'existence de la spécificité des fractions électrophorétiques des protéines hépatiques.

Les résultats obtenus sur les protéines sanguines prouvent l'existence du polymorphisme de cette catégorie de protéines. Il s'agit d'un polymorphisme par rapport au développement ontogénétique et aussi par rapport au sexe. On peut considérer que la fraction à mobilité électrophorétique moyenne se trouve en relation avec la maturation des gonades femelles.

L'étude du sang de l'hybride a prouvé que les caractéristiques électrophorétiques des protéines sanguines diffèrent des mâles et des femelles d'*Acipenser ruthenus*.

CONCLUSIONS

1. On a constaté l'existence du polymorphisme des protéines soluble des diverses catégories de cellules au cours du développement ontogénétique ainsi que par rapport au cycle de la réproduction chez *Acipenser ruthenus*.

2. En ce qui concerne les protéines solubles des oocytes chez l'esturgeon on a constaté des différences quantitatives et qualitatives au cours de la maturation de la gonade. Les stades III—IV et IV prouvent la continuité du même type de synthèse protéique.

3. Le polymorphisme des protéines hépatiques au cours du développement ontogénétique est significatif.

4. Le tableau électrophorétique des protéines sanguines est spécifique. On a constaté aussi l'existence du polymorphisme protéique par rapport à l'âge et au sexe. Chez les mâles adultes c'est la concentration de la fraction rapide qui est caractéristique, tandis que chez les femelles c'est la concentration de la fraction à mobilité électrophorétique moyenne.

5. Les protéines hépatiques et sanguines de l'hybride présentent des propriétés électrophorétiques différentes en comparaison d'*Acipenser ruthenus*, ce qui confirme la spécificité du tableau électrophorétique des protéines solubles.

BIBLIOGRAPHIE

1. Aida K., Phan-van-Ngan and T. Hibiya, (1973), Jap. Soc. Sci. Fish., **39**, 1091—1106.
2. Barlom John and George Ridgmay (1969), J. Fish. Res. Bd. Canada, **26** (8), 2101—2109.
3. Caloianu Iordăchel Maria, Doina Popov et coll. (1976). Rev. Roum. Biochim., **13** (3), 185—192.
4. Child S. E. (1973), Fish. Res. Bd. Canada, **30** *(5)*, 707.
5. Gray R. W. and J. A. McKenzie (1970), J. Fish. Res. Bd. Canada, **27** (7), 2101.
6. Heideman N. N., Seven R. et coll. (1975), J. Exp. Zool., **191** *(2)*, 253—260.
7. Johnson A. G., Fm. Utter (1970), J. Fish. Res. Bd. Canada, **27** *(5)*, 943.
8. McAllyster and coll. (1973), J. Fish. Res. Bd. Canada, **29** *(8)*, 1173.
9. Pech G. and coll. (1970), J. Fish. Res. Bd. Canada, **27** *(2)*, 951.
10. Suciu M. et Maria Caloianu Iordăchel (1977), Travaux du Muséum « G. Antipa «, *19*.

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cellulaire

XANTHINE OXIDASE IN THE FIRST STAGES OF DEVELOPMENT IN HAPLOID AND DIPLOID EMBRYOS OF *RANA RIDIBUNDA*

BY

D. SCRIPCARIU, R. MEȘTER, and M. BIVOLARU

The electrophoretic analysis of xanthine oxidase in non-fertilized eggs and in the course of growth, in haploid and diploid embryos of *Rana ridibunda* revealed the presence of an isoenzymic molecular polimorphism. Maternal isoenzymes (in non-fertilized eggs) and some others occurring during embryogenesis, were found. The persistence of the different material isoenzymes during ontogenesis is different in diploid embryos as against the haploid ones. In haploid embryos the embryonic isoenzymes seem to be scarce. The electrophoretic and quantitative differences of xanthine oxidase in haploid forms suggest a deficient genetic control.

Xanthine oxidase (xanthine : oxygen oxidoreductase EC 1.2.3.2), is a key enzyme in the metabolism of purine substances, as it catalyzes the xanthine oxidation at uric acid. The enzyme was found in many types of cells in vertebrates [1], [7], [12], [18] and microorganisms [6], [16]. The activity of this enzyme was also followed up in the ontogenesis of some vertebrates [14], [17], [19] as well as in the development of liver and kidney in birds [8].

Xanthine oxidase activity during the embryonic development of amphibians has been little studied. Barrett [2] studied the enzyme activity in the development of some organs in *Rana catesbiana*.

In the present paper we assumed the task to study the activity of xanthine oxidase in the non-fertilized eggs and in the first development stages of haploid and diploid embryos of *Rana ridibunda*, to gain some insight into the molecular mechanisms of the cellular differentiation associated with the morphogenetic processes.

MATERIAL AND METHODS

Frogs (*Rana ridibunda*) obtained from the lakes around Bucharest were used.

Maturation and ovulation, as well as the gynogenetic haploid embryos were obtained according to a method anteriorly described [18], [20]. The fertilized eggs developed in tap water at room temperature (18—20°C). The following embryonic stages were separated in haploid and diploid forms : 2 cells, 4 cells, morula, blastula, young gastrula, advanced gastrula, neurula, tadpole with caudate bud, swimming tadpole and actively nourishing tadpole. For comparison, the hepatic tissue of adult animals was also studied.

With a view of homogenization, the protective gel sheath of embryos was removed by mechanical means. The homogenization of all embryonic forms was performed with a Potter type homogenizer, in distilled water, at a dilution volume of 10 embryos/ml water. After 2 hours of protein extraction at cold, the homogenates were centrifugated at cold, at

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7 000 r. p. m., for 10 min. The supernatants obtained after centrifugation were electrophoretically and spectrophotometrically analyzed.

The electrophoretic separation of the different proteic fractions with xanthine oxidase activity was made on polyacrylamid gel, disk-electrophoretic system, according to the technique described by Davis [9], in discontinuous buffer solution [15]. The gels were embedded for 4 hours, at 37°C, in a complex medium consisting of: phosphate buffer 0.05 M, pH 7.5, xanthine 20 μ mole and artificial electron acceptors 1 mg/ml (phenazinmetosulphate and nitrotetrazolium blue). The numerotation of proteic bands was made from the anode to the cathode according to Webb [22].

The activity of xanthine oxidase was determined spectrophotometrically at 550 nm, following up the amount of reduced cytochrome c formed. The activity of xanthine oxidase was expressed by micromoles of cytochrome c reduced /g protein/ min, or in U/g protein/min.

The protein content in the total proteic extracts of the embryonic forms studied were determined spectrophotometrically at 260 and 280 nm.

RESULTS

Electrophoretic study. The electrophoretic spectra of xanthine oxidase in the total proteic extracts of the embryonic forms studied (n and 2n) are presented in Fig. 1. Two categories of isoenzymic fractions are obtained; a first category includes the isoenzymes corresponding, as to their position in gel, to those of the electrophoregram of the non-fertilized egg, called maternal isoenzymes (laid during the growth and vitellogenesis period of the oocytes); the second category, with embryonic enzymes, which occur in different stages of ontogenesis and have no equivalent in the enzymic fractions in the extracts of the non-fertilized eggs.

The electrophoregram of the non-fertilized egg reveals 9 bands with enzymic activity, the first 5 fractions of which are found in all embryonic stages studied (maternal isoenzymes). The first four also appear in the hepatic tissue of the adult animal. The isoenzymic fractions 6 and 7 persist till the gastrula stage, and fraction 8, till the stage of neurula. During the development of diploid embryos, a few bands with enzymic activity and a short life, are described: band 9 from advanced gastrula remaining active up to the stage of hatched tadpole; bands 2 and 7, in the swimming tadpole. In the stage of nourishing tadpole, enzymic fraction 7b is noted, having its equivalent in the zymogram of the liver of adult animals.

The electrophoretic spectrum of the hepatic tissue in adults animals reveals 4 maternal isoenzymic molecular fractions and 2 isoenzymes with particular electrophoretic properties.

In haploid embryos, the isoenzymic fractions 1, 3, 8 and 9 are maintained in all the studied stages; bands 2 and 6 persist up to the stage of caudate tadpole. The electrophoretic analysis of the enzymic molecular forms of xanthine oxidase in haploid embryos, shows 2 isoenzymes with characteristic properties (1*a* and 4*a*). These two bands are characteristic of the haploid type in embryos (Fig. 1).

The spectrophotometric study. In parallel, a quantitative study of the xanthine oxidase activity was carried out in the nonfertilized eggs and embryonic forms n and 2n investigated (Fig. 2).

As shown in figure 2, the activity of xanthine oxidase in nonfertilized eggs is relatively small (2.17 U/g protein/min). After fertilization,

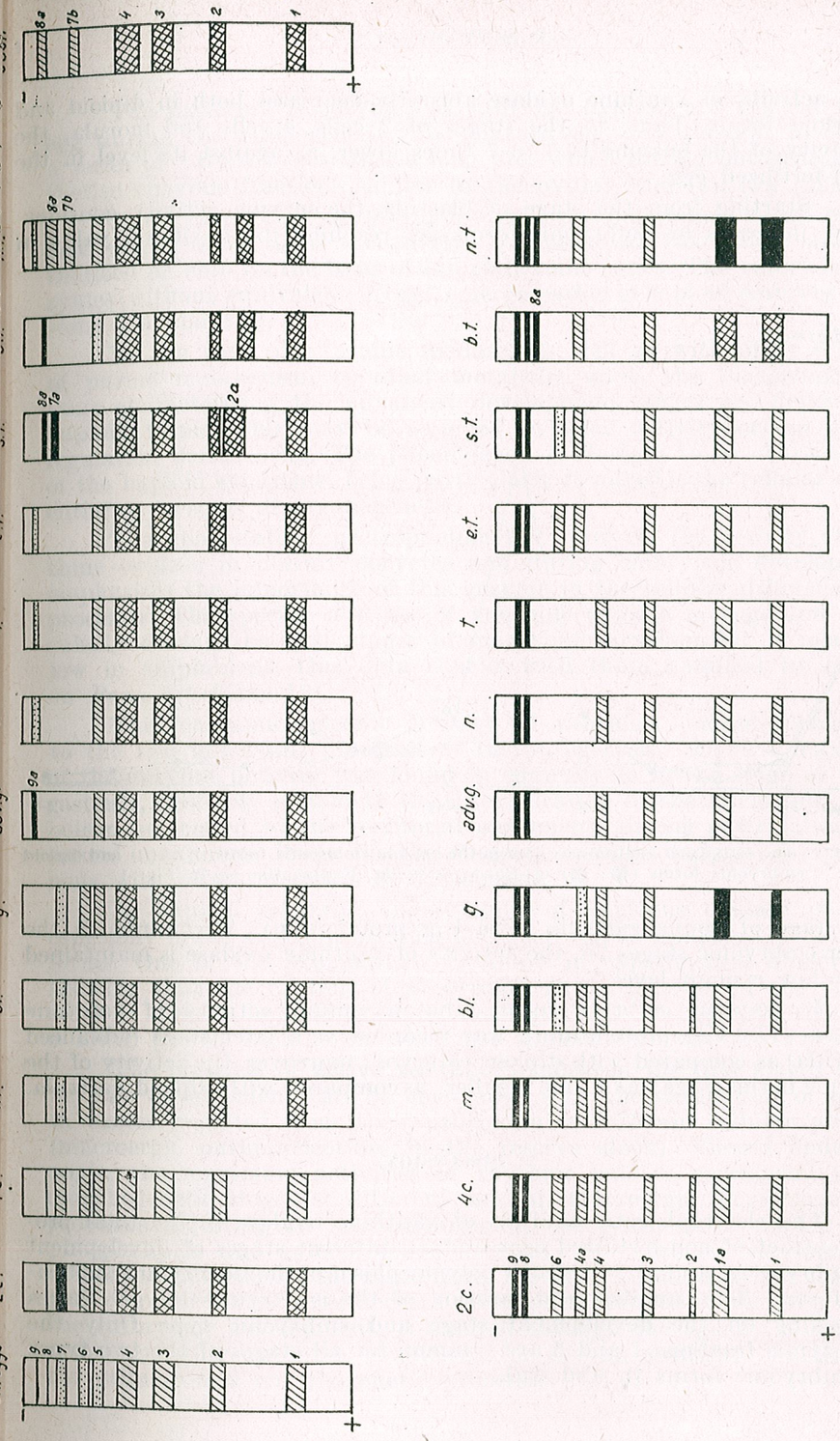

Fig. 1. — The electrophoretic spectrum of xanthine oxidase in non-fertilized eggs and haploid embryonic forms (*n*) and diploid embryonic forms (2n) of *Rana ridibunda*. un. eggs, unfertilized eggs; 2 c., 2 cells; 4 c., 4 cells; m., morula; bl., blastula; g., young gastrula; adv. g., advanced gastrula; n., neurula; t., tadpole with caudate bud; e. t., eclosed tadpole; s. t., swimming tadpole; b. t., tadpole with mouth; n. t., nourishing tadpole.

the activity of xanthine oxidase abruptly decreases both in diploid and haploid forms. Thus, in the stages of 2 cells, 4 cells and morula, the activity of the enzyme is 5 to 7 times lower, as against its level in the non-fertilized egg.

Starting from the stage of blastula, the enzyme activity considerably increases in embryonic forms 2n, reaching its maximum value in

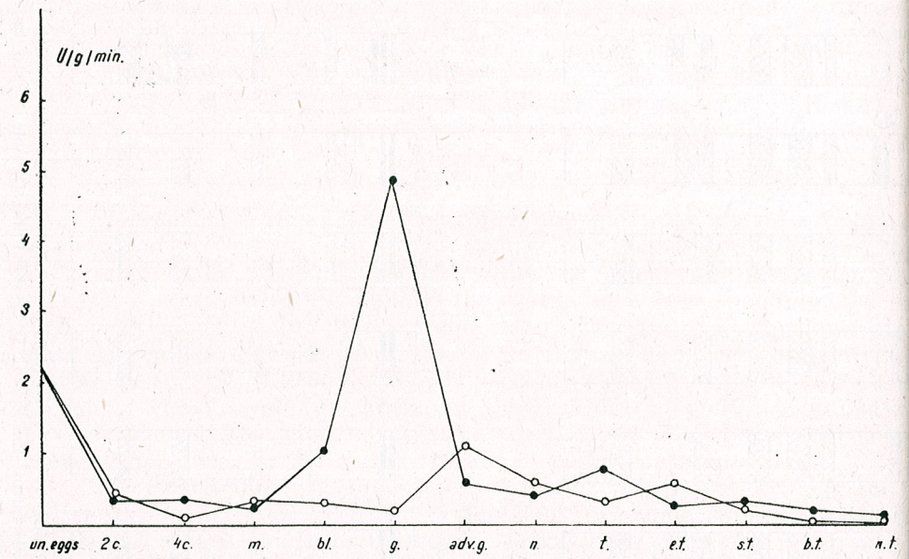

Fig. 2. — The activity of xanthine oxidase in the haploid embryonic forms (○—○) and diploid embryonic forms (●—●) of *Rana ridibunda*. Explanation as in Fig. 1.

the stage of young gastrula (4.86 U/g protein/min). Afterwards, in the other embryonic stages 2n, the activity of xanthine oxidase is maintained at a low enough level.

In haploid embryos forms, the maximum activity of xanthine oxidase (1.10 U/g protein/min), was recorded at a later stage (advanced gastrula) as compared with diploid embryos; moreover, the activity of the enzyme in this stage was 4 time smaller, as compared with diploid gastrula.

DISCUSSION

The electrophoretic analysis of xanthine oxidase in the total proteic extract of non-fertilized eggs and at different stages of development of embryos 2n and n, revealed a polymorphism of the isoenzymic molecular forms. The number and position of the isoenzymes in gel varies depending on the development stage and embryonic type. Only the isoenzymic fractions 1 and 3 are common for all stages of the two types of embryonic forms (n and 2n).

The maternal isoenzymes of xanthine oxidase are stocked in the egg during the vitellogenesis. The mature eggs are thought to contain a series of RNA types with a long life, which during ontogenesis synthetize enzymic fractions similar to the ovular ones (4), (5), (21), (23).

In the cases of isoenzymic molecular forms maintained in all embryonic stages, adult tissue inclusively, it is assumed that these are synthetized *de novo* on the basis of the embryonic genes. The embryonic enzymes (without equivalent in egg), emerge earlier in haploid embryos (fractions 1 a and 4 a).

In the case of xanthine oxidase, as well as with other enzymes of purine metabolism, or other metabolic ways, the isoenzymic fractions characterizing the advanced development stages are deficient in haploid forms. This may be assigned to some activity or/and genetic regulation defficiencies. The phenomenon is thought to be characteristic of the haploid syndrome, being particularly involved in the cellular differentiation process of organogenesis.

The quantitative spectrophotometric study of the activity of xanthine oxidase in mature oocyctes and during embryonic development, emphasizes the importance of this enzyme in the cellular differenciation processes. The specific activity of xanthine oxidase in eggs and particularly during the first stages of embryonic development is relatively low in amphibians. Our data concur with those obtained by Barrett on *Rana catesbiana* [2].

The maximum specific activity of xanthine oxidase corresponds to the two important processes of the ontogenesis; the first maximum of the enzymic activity was found in embryos 2n in the stage of young gastrula, probably related to the intensification of the nucleotids' metabolism associated to the territorial movements during gastrulation; the second maximum, lower (in the stage of caudate tadpole) is synchronous with offset processes of ontogenesis.

In haploid embryos, the activity of xanthine oxidase is lower, especially in the stages of blastula and gastrula, as compared with diploid forms. One of the explanations is found in experimental observations showing that the rhythm of absorption and utilization of the vitelline stock is lower in haploid embryos. Consequently, the haploid forms show a lower proteic metabolism than the diploid embryos [3], [10], [11].

The presence of active xanthine oxidase in the first stages of embryonic development can be correlated with the catabolism of purines in excess, resulted from their utilization in different cellular processes (macroergic purinnucleotides in the reserve stock). Viewed under this angle, the maximum activities of xanthine oxidase correspond to two morphogenetic stages of different use of macroergic purinnucleotides, processes requiring an intensification of the synthesis of nucleic acids, phospholipids and gylcoproteins, necessary to establish the specialized cellular structures. At the same time, the metabolism of mononucleotides and of other many intermediate metabolism products emerging during the embryonic development, is assured through the agency of some enzymes granting optimum conditions for the intracellular reactions to the cellular and morphogenetic processes.

REFERENCES

1. Avis P. G., Bergel, Bray R. C., 1965, J. Chem. Soc., **26**, 1100.
2. Barrett D., 1964, Develop. Biol., **10**, 289.
3. Brachet J., 1954, Arch. Biol., **65**, 1.
4. Brown D. D., 1967, J. Exptl. Zool., **157**, 101.
5. Brown D. D., Littna E., 1964, J. Mol. Biol., **8**, 669.
6. Bradshaw W. H., Barker H. A., 1960, J. Biol. Chem., **235**, 12.
7. Brumby P. E., Massey V., 1963, Biochem., J., **89**, 46.
8. Croisille J., 1962, C. R. Acad. Sci., **254**, 2253.
9. Davis J. B., 1964, Ann. N. Y. Acad. Sci., **121**, 404.
10. Fox H., 1971, J. Embryol. exp. Morphol., **26**, 81.
11. Fox H., Hamilton L., 1964, Experientia, **20**, 289.
12. Klenow H., Emberland R., 1955, Arch. Biochem. Biophys., **58**, 276.
13. Meşter R., Scripcariu D., 1976, Rev. roum. Biol.-Zool., **21**, 35.
14. Moog F., 1958, in *Enzymes formation and growth in embryonic nutrition*, ed. Rudnick P., Chicago Univ. Press, Chicago.
15. Ornstein L., 1964, Ann. N. Y. Acad. Sci., **121**, 322.
16. Rabinowitz J. C., Barker H. A., 1956, J. Biol. Chem., **218**, 161.
17. Rossi F., 1955, Z. Anat. Entw. Gesch., **117**, 36.
18. Roussos G. G., 1963, Biochem. Biophys. Acta, **73**, 338.
19. Roussos G. G., 1967, in *Methods in enzymology*, ed. Colowick S. P., Kaplan N. O., New York — London, **13**.
20. Scripcariu D., Meşter R., 1973, St. cercet. Biol-Zool., **25**, 223.
21. Wallace H., Elsdale T. R., 1963, Acta Embryol., Morphol. Exptl., **6**, 275.
22. Webb E. C., 1964, Experientia, **50**, 592.
23. Wright A. D., 1968, J. Exptl. Zool., **167**, 197.

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ROLE OF PARATHYROID GLAND IN ESTROGEN-INDUCED HYPERCALCEMIA OF THE FROG, *RANA TIGRINA*

BY

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Although involvement of parathyroid gland in calcium metabolism has been studied by a number of workers, using crude extract of parathyroid gland and parathyroidectomy [4, 9], very little is known about its role in estrogen-induced hypercalcemia. In egg laying vertebrates, plasma calcium level increases during breeding season [12, 14], mostly due to elevated level of estrogen. Similarly exogenous estrogen is also shown to increase the circulating calcium level [12]. Yet it is not known, whether parathyroid is involved in the estrogen-induced hypercalcemia. Therefore, present study was carried out to elucidate the function of parathyroid gland.

MATERIAL AND METHOD

Adult male frogs, *Rana tigrina* used for the present investigation were obtained from local commercial source. These were acclimatized for a week to laboratory conditions. Experimental animals were kept in aquaria with sufficient water. Owing to individual differences in appetite, food was not offered during experiment. Temperature during the course of experiment was 28—32°C. Oil suspension of estrodial dipropionate (Ovacyclin P, Ciba Ltd., 25 μg/100 g/day) was injected intramuscularly for 20 days. Control animals received equivalent amount of vehicle. Animals from both groups were slain by excess of ether anaesthesia after 10 and 20 days and blood samples were obtained from vena cava. Plasma was separated and calcium was estimated by using flame photometer.

Parathyroid gland with adjacent tissue was removed and fixed in Bouin's fluid for 24 hours. Paraffin sections of 6 μm thickness were stained with haematoxylin-eosin. Cell and nuclear diameter were measured by using Lanameter (PZO, Poland) and nuclear cytoplasmic ratio (N/C) was determined. Under suitable magnification number of cells were measured on Occulometer and total population of cells per gland (Cells/gland) were calculated. Gland volume was obtained as described previously [7]. Student's t was used for the statistical procedure.

OBSERVATIONS

The plasma calcium level in the estrogen-treated animals showed an increase as compared to those in the control. The average plasma calcium level in the animals treated for 10 and 20 days were 10.60 ± 0.42 and 12.20 ± 0.67 mg/100 ml respectively (Table 1).

Animals treated with estrogen showed degenerative changes in the parathyroid accompanied by atrophic parenchymal cells with shrunken nuclei and cytoplasm (Fig. 1). The degenerative changes, though present were less marked in the animal sacrificed after 10 days. Nuclear cytoplasmic ratio, total population of cells and gland volume were decreased significantly after estrogen treatment (Table 1).

Table 1

Effect of estrodial dipropionate on plasma calcium level and parathyroid gland of the frog, Rana tigrina

Treatment	Days	Plasma calcium (mg/100 ml)	Parathyroid gland		
			N/C Ratio	Cells/Gland	Volume of gland (mm^3)
Initial control		8.40±0.60(12)			
Control	10	7.80±0.52(4)	1/2.0	700000±5000	0.32±0.02
Estrodial-dipropionate	10	10.60±0.42*.(5)	1/1.8	620000±4500	0.28±0.03
Control	20	8.00±0.32(4)	1/1.9	680000±7000	0.31±0.01
Estrodial-dipropionate	20	12.20±0.67*(5)	1/1.4*	520000±4200*	0.23±0.02*

Values are shown ±SE.
In parenthesis figures indicate number of animals
* P <0.001

DISCUSSION

Significant higher plasma calcium level obtained in the frog *Rana tigrina* following estrodial treatment is in well agreement with the previous reports on several egg-laying vertebrates [1, 5, 14, 3, 10].

The role of parathyroid gland in egg-laying vertebrates during estrogen-induced hypercalcemia is not yet clearly understood. Presently, two hypotheses regarding the role of parathyroid gland in the estrogen-induced hypercalcemia are in vogue. Benoit *et al.* [2] demonstrated the importance of parathyroid gland in estrogen-induced hypercalcemia, as parathyroidectomized ducks did not show any effect of calcium level after estrogen treatment. Similarly Fleming and Meier [8] were unable to demonstrate any change in serum calcium level following parathyroid hormone injection in the female, *Fundulus kansae* unless the animals were pretreated with estrogen. On the other hand, many reports suggest that parathyroid glands are not essential for estrogen-induced hypercalcemia [12, 11, 10, 13]. The results of the present investigation indicate that parathyroid glands are not essential in the estrogen-induced hypercalcemia, as the parathyroid appeared inactive after estrogen-induced hypercalcemia. Similar results were also obtained in the lizard, *Uromastix hardwickii* after experimentally induced hypercalcemia [6]. However, the study on effect of estrogen in the absence of parathyroid gland, presently under investigation, may provide final confirmation regarding the involvement of parathyroid gland in estrogen-induced hypercalcemia in *Rana tigrina*.

CONCLUSIONS

Injections of the estrodial dipropionate brought hypercalcemia accompanied with degenerative changes in the parathyroid glands of the frog, *Rana tigrina*. The results suggest that the parathyroid gland is not essential for hypercalcemia produced after estrogen treatment.

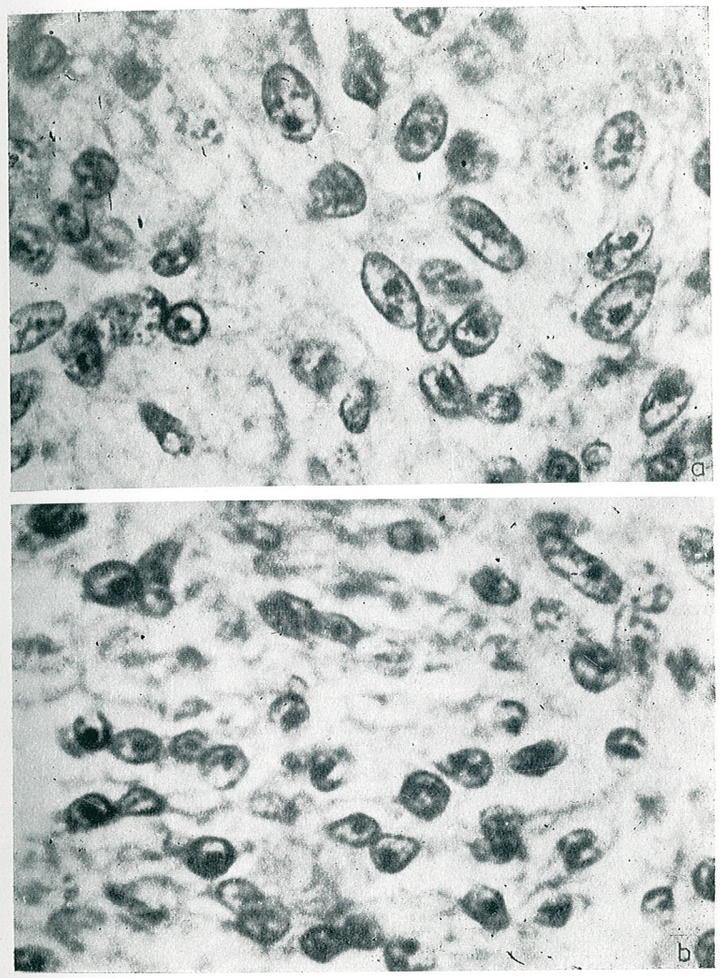

Fig. 1. — Parathyroid gland of frog *Rana tigrina*.
a, Part of the gland from control animal; b. Part of the gland from estrogen-treated animal. Note the degenerative changes. (Haematoxylin-eosin, ×1000).

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BIBLIOGRAPHY

1. Bailey, R. E., J. Exp. Zool., 1957, **136**, 455–469.
2. Benoit, J., Stricker, P., Fabiani, G., 1941, Comp. Rend. Soc. Biol., **135**, 1600–1602.
3. Clark, N. B., 1967, Comp. Biochem. Physiol. **20**, 823–834.
4. Cortelyou, J. R., McWhinnie, D.J., 1967, Am. Zool. **7**, 843–855.
5. Dessauer, H. C., Fox, W., 1959, Am. J. Physiol. **197**, 360–366.
6. Dubewar, D. M., P. D. Thesis submitted to Nagpur University, 1976.
7. Dubewar, D. M., Suryawanshi, S. A., Z. mikrosk. anat. Forsch. (In press).
8. Fleming, W. R., Meier, A. H., 1961, Comp. Biochem. Physiol., **2**, 1–7.
9. Greep, R. O., *Comparative Endocrinology*, Academic Press, New York, 1963.
10. Oguro, C., Uchiyama, M., Ann. Zool. Jap., 1972, **45**, 214–217.
11. Polin, D., Sturkie, P., Endocrinol., 1958, **63**, 177–182.
12. Simkiss, K., *Reproductive Physiology*, Chapman and Hall Ltd, London, 1967.
13. Urist, M. R., Deutsch N. M., Pomerantz, G., McLean, F. C., Amer. J. Physiol., 1969, **199**, 851–855.
14. Urist, M. R., Schjeide A. O., J. Gen. Physiol., 1961, **44**, 743–756.

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INSULIN INFLUENCE ON THE K^+-INDUCED DEPOLARIZATION AND REPOLARIZATION OF STRIATED MUSCLE FIBER MEMBRANE IN DIFFERENT CONDITIONS OF pH AND CONCENTRATION

BY

ȘT. AGRIGOROAEI and MARGARETA CRĂCIUN

The experiments were performed on single muscle fibers of the frog by the intracellular glass microelectrode technique. Insulin increases the depolarization by 30 mM K^+-Ringer (pH= 7.2). But the repolarization in normal Ringer is slowed down. On the other hand, at pH= 6, insulin decreases the amplitude of K^+-depolarization and increases the repolarization. These results are explained as modifications of the passive permeability, on the basis of the insulin interaction with membrane Ca^{++} during the phase transition of the phospholipid micella. The phenomenon is evidently influenced by the medium pH.

In a previous paper [1] we have shown that the hyperpolarizing effect of insulin on the resting membrane in a normal external ionic medium most likely resulted from its stimulative action on the active Na^+ transport, the hormone being not involved in a change of the membrane passive permeability.

However, there are also data describing some effects of insulin on the electrical charge of the membrane when the ionic ratios in the extracellular medium are modified, and these results raise in a way or another, the question of the interference of active transport changes with changes in permeability. Thus, Otsuka and Ohtsuki [13] investigated the response of rat isolated diaphragm fibers to variations in external K^+ concentration in absence of insulin, using a preparation obtained from animals fed a K^+-free diet. In such animals the K^+ concentration in serum is greatly reduced. As regards the internal K^+ concentration (in muscle fibers) the opinions are contradictory. The above cited authors as well as Offerijns [12] claim that the intracellular K^+ concentration is almost normal (the permeability to K^+ is decreased and therefore the loss of intracellular K^+ reduced).

Many earlier and more recent investigations showed that the absence of external K^+, both *in vivo* (by K^+-free diet [4. 5, 7] and *in vitro* (by incubation of muscle fibers in a medium devoid of K^+) [2, 6, 15, 16, 17] resulted in an enrichment of the muscle fiber in Na^+.

It was also shown [3, 14] that the increase of intracellular Na^+ may result from the inhibition of Na^+ pump (by decrease of extracellular K^+). As a direct consequence of the passive influx of Na^+ which would replace the loss of intracellular K^+, the concentration gradient

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of K^+ is increased in order to maintain the osmolarity. Apart from these contradictory data regarding the internal K^+ concentration, it is worth mentioning that Otsuka and Ohtsuki [13] found that muscle fibers obtained from animals fed a K^+-free diet responded normally to variations of the external K^+ concentration (in experimental conditions) in absence of insulin. Thus, a decrease of the external K^+ substituted for Na^+ resulted in a hyperpolarization of the membrane, whereas an increase of external K^+ on account of Na^+ produced a typical depolarization — as in normal preparation.

However, different responses were obtained with insulin. In an external medium with normal K^+ concentration the membrane becomes hyperpolarized (the well known insulin action on the resting membrane in normal external conditions). If the external K^+ was slightly decreased, a reduced membrane hyperpolarization took place also, which rapidly decreased, replaced by a depolarization of the membrane more pronounced as the external K^+ decreased.

On the contrary, if the external K^+ concentration is slightly over normal, the hyperpolarizing action of insulin is enhanced (compared to that obtained with normal external K^+ concentration). Subsequent increase of the external K^+ up to two-fold the normal value resulted in a gradually attenuated hyperpolarization. When the external K^+ was further increased, a depolarization of the membrane occurred, whereas increasing the external K^+ concentration over 30 mM, this depolarization was similar to that in a high K^+ medium without insulin. Pointing out the depolarizing effect of insulin at low external K^+ concentration, the authors concluded that this would result from an insulin action of increasing the membrane permeability to Na^+ (in conditions of an admissible fall in permeability to K^+). It is known that in a low external K^+ medium or a K^+-free medium the active Na^+ efflux is inhibited and Moore [11] reported that in such conditions insulin is no longer able to stimulate the Na^+ pump by itself.

Therefore, the reduced hyperpolarization still observed at a slight decrease of external K^+ in the presence of insulin, could not be attributed to the hormone itself, this being in our view, the direct expression of the change of Ca^{++}/K^+ ratio in the extracellular medium. The effect is not too strong, particularly because of its overlapping with the typical depolarizing effect of insulin in these conditions. In turn, the effect of insulin could not be accounted for only by a single factor since :

— insulin does not depolarize the membrane at low external K^+ concentrations in preparations obtained from animals fed a normal K^+ diet (therefore the decrease of external K^+ is not the only cause of the effect) [12] [13];

— if the external K^+ is brought at a normal value (in preparations from animals fed a K^+-free diet) a hyperpolarization of the fibers is observed instead of a depolarization (the decrease of external K^+ is therefore a condition for the depolarizing effect of insulin).

Thus, it should be admitted that the depolarization produced by insulin is due to an extracellular factor on one hand and to an intracellular factor on the other (or related to the membrane itself).

As regards the effect of insulin at a slight increase of extracellular K^+ (more pronounced hyperpolarization), this is fully explained if one considered the fact that in these conditions both the hormone and K^+ stimulated the activity of Na^+ pump. When the threshold of the pump is exceeded, the typical action of depolarization by increased external K^+ becomes prevalent.

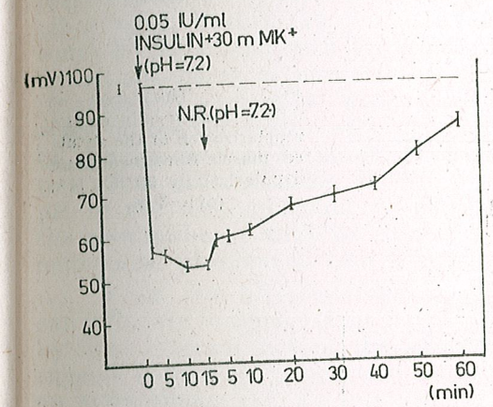

Fig. 1. — The effect of 0.05 I. U. /ml insulin on the depolarization of the striated muscle fibre membrane by 30 mM K^+ (pH= 7.2).

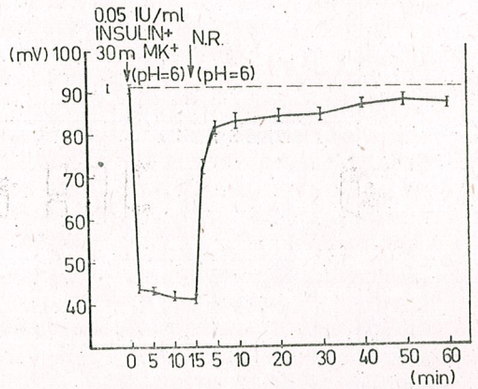

Fig. 2. — The effect of 0.05 I. U./ml insulin on the depolarization of the striated muscle fibre membrane by 30 mM K^+ (pH= 6).

However, at an external K^+ concentration up to 30 mM even the K^+-induced depolarization seems slightly influenced by insulin and it is worth noticing that in preparations obtained from K^+-deficient animals insulin (in this range of K^+ concentration and normal pH) tends to slightly reduce the magnitude of depolarization.

On the contrary, in our experiments carried out on normal animals we found that at a pH=7.2 and K^+ concentration of 30 mM, insulin slightly enhanced the depolarization (Fig. 1). On the other hand, the recovery from depolarization (by replacing the preparation in normal Ringer without insulin) is stronger influenced, namely by slowing down the phenomenon especially in its initial phase (Fig. 1). Evidently, since the washing Ringer contained no insulin, this effect is to be attributed to the insulin bound to the membrane during the depolarization (we have shown that a pH=7.2 maintained insulin bound for a long time).

Furthermore, this effect of insulin on the membrane repolarization was also found when the fibres were submitted to a depolarization with 30 mM K^+ in absence of insulin, the hormone being added together with the washing Ringer (Fig. 2). This effect seems even more pronounced in these conditions.

Our experiments also showed that influence of insulin greatly depended upon the pH of the medium. At a pH=6 we found a slight reduction of the magnitude of K^+-induced depolarization (as in K^+-

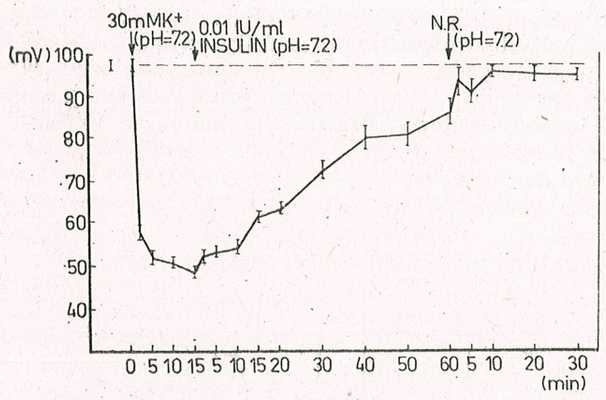

Fig. 3. — The effect of 0.01 I.U/ml insulin on the repolarization of the striated muscle fibre membrane depolarized by 30 mM K^+ (pH= 7.2).

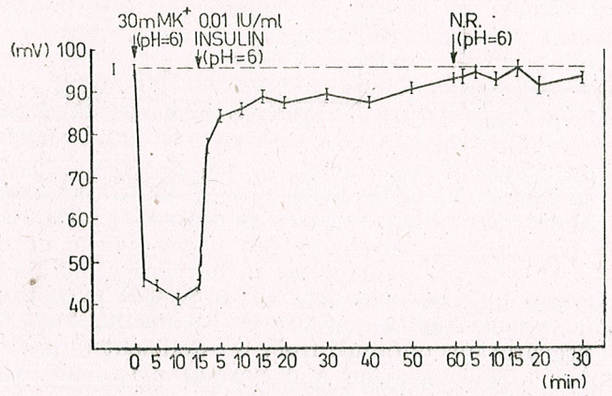

Fig. 4. — The effect of 0.01 I.U./ml insulin on the repolarization of the striated muscle fibre membrane depolarized by 30 mM K^+ (pH= 6).

deficient animals) and a reverse effect on the repolarization, that is an acceleration, especially in its initial phase (Fig. 3).

The speeding up of the repolarization also occurred when insulin was added in the washing Ringer during the repolarization and not during the depolarization (the effect being even more pronounced (Fig. 4)).

Since we consider the high K^+-depolarization as a result of a phase transition from Ca^{++} laminar micella of the external layer to K^+ globular micella and the repolarization as a phase transition in an opposite direction (therefore rebinding Ca^{++} in the structure), all the observations regarding the action of insulin on the high K^+ — depolarization suggest a certain interaction between insulin and Ca^{++} with a release and rebinding of the latter to the membrane, interaction which is in turn very clearly influenced by the pH of the medium (at an alkaline pH Ca^{++} rebinding is prevented or delayed, whereas at an acid pH it is facilitated).

There are some data available on the possibility of interaction between insulin and Ca^{++} derived from studies made on model systems (artificial membranes). Kafka and Pak [8, 9] pointed out the ability of insulin to compete with Ca^{++} for free phosphate groups of the monolayer constituents (stearyl phosphate) at the water-air interface. However, as it was shown in other cases, the results derived from model systems cannot be applied similarly to the biological membranes. On the other hand, Kreutz [10] indicated the possibility that some substances could be associated with the lipidic leaflets of the membrane, no phase transitions being determined by this associations. Thus, it is possible that insulin associates with the phospholipids of the external layer even in resting state, without modifying the membrane permeability by slowing down or facilitating the phase transitions imposed by other factors. The differentiation of these actions of insulin according to the pH of the medium could be correlated either with insulin binding to the membrane or with its suprastructural organization in more or less monomers, phenomena also shown to depend on the extracellular pH.

From our investigations it is possible to conclude that insulin can influence to some extent the changes in passive permeability produced by some external factors, i. e. the variation of ionic ratios. These effects suggest a possible interaction with Ca^{++} which depends on its action on extracellular pH. To solve the intimate mechanisms of these actions of insulin more detailed investigations in this direction are needed.

REFERENCES

1. Agrigoroaei St., Crăciun Margareta, 1976, A II-a Conf. Biol. Cel. București, 12—14 febr. 1976, 41.
2. Carey M. J., Conway E. J., 1956, Biochem. J., **64**, 41.
3. Cier J. F., 1961, J. Physiol. (Paris), **53**, *4*.
4. Conway E. J., Hingerty D., 1948, Biochem. J., **42**, 372.
5. Cooke R. F., Segar W. E., Cheek D. B., Coville F. E., Darrow D. C., 1952, J. Clin. Invest., **31**, 798.
6. Desmedt J. E., 1953, J. Physiol. (Lond.), **121**, 191.
7. Heppei L. A., 1939, Amer. J. Physiol., **127**, 385.
8. Kafka M. S., Pak C. Y. C., 1969 a, J. Gen. Physiol., **54**, 134.
9. Kafka M. S., Pak C. Y. C., 1969 b, Biochim. Biophys. Acta, **193**, 117.
10. Kreutz W., 1972, Angew. Chem., **13**, 597.
11. Moore R. D., 1972, J. Physiol. (Lond.), **232**, *1*, 23—45.
12. Offerijns F. G. J., Westerink D., Willebrands A. F., 1958, J. Physiol. (Lond.), **141**, 377.
13. Otsuka M., Ohtsuki I., 1970, Amer. J. Physiol., **219**, *5*, 1178.
14. Sachs J. R., Conrad M. F., 1968, Amer. J. Physiol., **215**, 795.
15. Shaw H. F., Simon S. F., 1955, Nature, **176**, 1031.
16. Steinbach H. B., 1940, J. Biol. Chem. **133**, 695.
17. Steinbach H. B., 1951, Amer. J. Physiol., **167**, 284.

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ACTION OF METHYLANDROSTENEDIOL ON THE BIOSYNTHESIS OF GLUCOCORTICOID HORMONES IN ADULT AND YOUNG FEMALE RATS

BY

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A chronic treatment with methylandrostenediol on both intact and pregnant rats was applied. The activity of adenyl cyclase, 3β-steroid-dehydrogenase and incorporation of (2—^{14}C)) acetate into free cholesterol and glucocorticoid hormones (corticosterone + hydrocortisone) was studied in adult intact female rats and in 21-day-old young ones born by females treated during pregnancy. The increase of activity of enzymes in zona fasciculata and the stimulation of the *de novo* synthesis of glucocorticoid hormones was observed in both cases.

Studies were undertaken with a view to elucidating the biochemical mechanisms, particularly that of anabolic steroid interaction with the control mechanisms of glucocorticoid biosynthesis in rat adrenal cortex. The effect of various androgen hormones and anabolic steroids on adrenal glands has been studied by Korenchevschy [6], Krüskemper [7], Selye [11], Abraham [1] and Hasan [4]. It is suggested that androgen administration causes depletion of hormone storage in normal and ACTH-treated male rats [9, 11], and it has been shown both experimentally and clinically that treatment with anabolics may depress the functional activity of the adrenal glands. In contrast with these observations we demonstrated the stimulation of *de novo* synthesis of glucocorticoid hormones in intact female rats and in the young ones born by females treated during pregnancy.

MATERIAL AND METHODS

Intact adult female Wistar rats (3—4 months old) were treated during 28 days with a total dose of 175 mg 17α-methyl-androsta-4-ene-3β, 17β-diol (Madiol, U. M. B., Bucharest) dissolved in milk and administered directly into oral cavity. Pregnant rats were treated with about the same dose and the 21-day-old young animals born by these females were examined. Control animals received milk under the same conditions. The animals were killed by cervical dislocation 1 hour after i. p. injection with 2 μCi (2-^{14}C) acetate (isotonic solution). The adrenals were removed quickly and examined histochemically and biochemically.

Adenyl cyclase activity (ADC) was studied with a method described by Howell and Whitfield [5]; 3β-steroid-dehydrogenase (STDH) with a technique described by Baillie et al. [2]. For the biochemical studies the adrenals were homogenized in methanol and glu-

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cocorticoids were eluted as described by Bernauer and Schmidt [3]. The crude extracts were eluted with ethyl acetate-dichlormethane (1 :1 v/v) and applied on silica gel F_{254} fluorescence plates (thickness : 200 μm) (E. Merck, Darmstadt). Zones of silica gel (1 cm^2) were scraped from the plates and eluted with methanol. Aliquots of the methanol extracts were counted in Bray's solution using BF-5003 type liquid scintillation spectrometer. The exact position of steroids and sterols on chromatogrammes was determined in ultraviolet light and with a tetrazolium blue coloration technique [8].

RESULTS AND DISCUSSION

ADC and STDH enzymes are involved in steroid hormone biosynthesis. ADC stimulates cyclic AMP formation, which enhances steroidogenesis. Cyclic AMP is the obligatory mediator of the steroidogenic action of ACTH on adrenal cortex. Recently it has been demonstrated that cyclic AMP induces synthesis of "cholesterol carrier protein" [12]. This protein increases the rate of delivery of cholesterol to a locus on the cytochrome P-450 system at which side-chain cleavage occurs. STDH catalyses the oxidation of steroid precursors into glucocorticoid hormones. The augmentation of both enzymes activities were observed by intact adult females and by young ones born by treated pregnant females, as resulted from figure 1. The enhancement of enzymes activities takes place especially in zona fasciculata, where the glucocorticoid hormones are formed.

Furthermore, we conducted experiments for the elucidation of the action of methylandrostenediol on the incorporation of the (2—^{14}C) acetate and cholesterol into glucocorticoids. From radiochromatogrammes (Fig. 2) resulted that radiocarbon is incorporated above all into glucocorticoid fraction, meanwhile the specific radioactivity of free cholesterol remained unchanged. The concentration of free cholesterol (Fig. 3) decreased significantly in the treated groups, which suggests an elevated rate of utilization of this substance for glucocorticoid biosynthesis.

These results showed that methylandrostenediol stimulates the activity of steroidogenic enzymes and incorporation of acetate and cholesterol into glucocorticoids, but has no direct influence on the rate of cholesterol biosynthesis in adrenals. The effect of methyl-androstenediol during pregnancy is exerted on foetal adrenal gland, manifested by a higher level of adrenal synthetic capacity, which may be observed also in 21-day-old rats, however a postembrional treatment was not continued. The effect of methylandrostenediol on female rats and the different reaction of female adrenal gland may be explained by specific answer of nervous and endocrine systems of female animals which differ from the males.

Aknowledgement. We are thankful for the skilful technical assistance of Ilyés I., Mihăeș V. and Brie E.

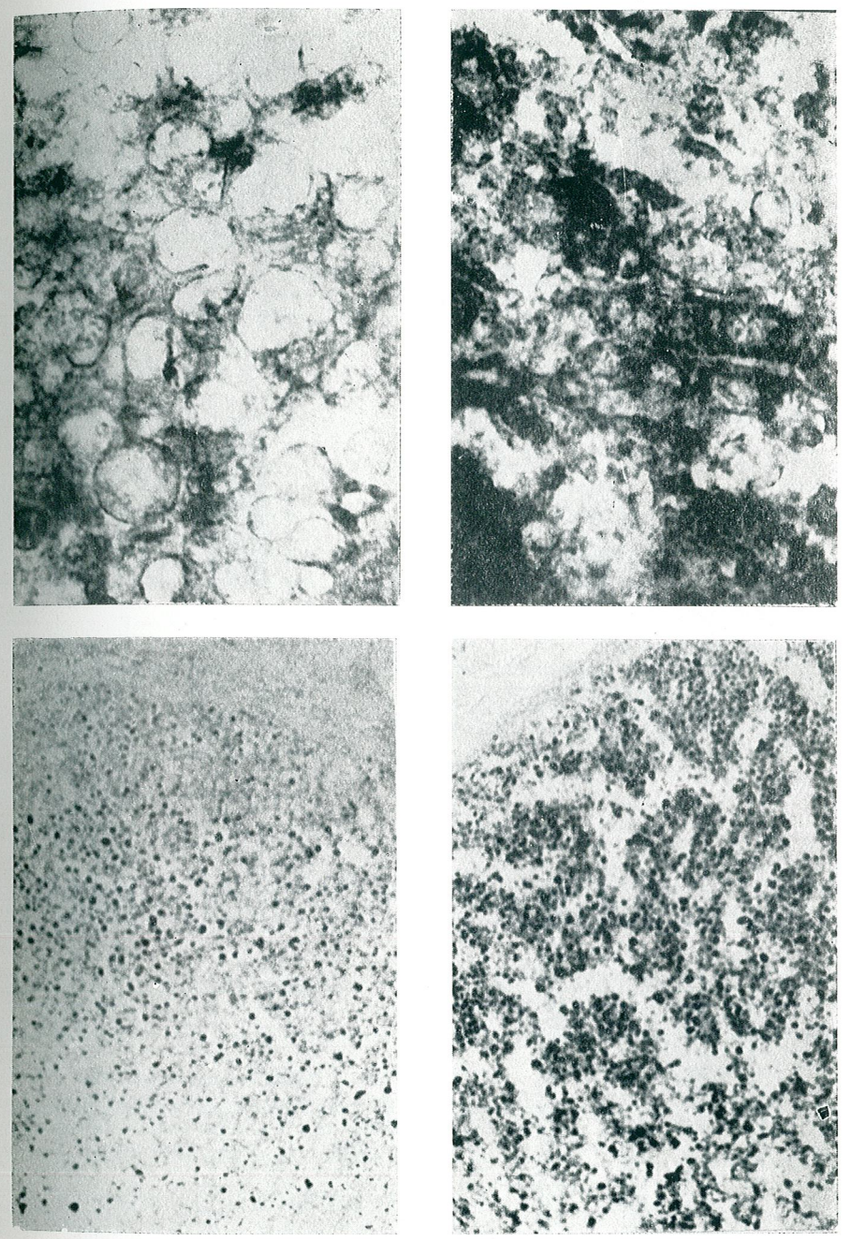

Fig. 1. — Activity of ADC and STDH in zona fasciculata of adrenal cortex by normal (*a* respectively *c*) and methylandrostenediol treated adult female rats (*b* respectively *d*).

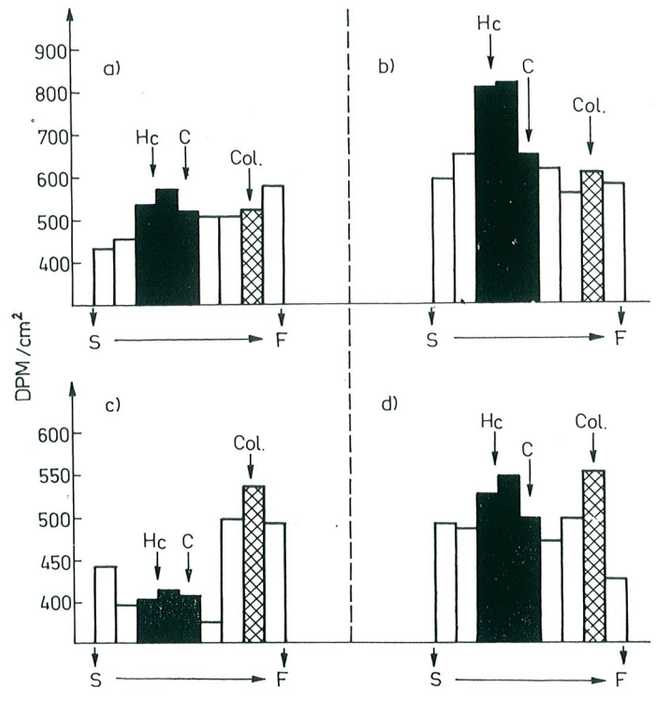

Fig. 2. — Radiochromatogrammes of steroid extracts obtained from adrenals of normal adult rats (*a*) and treated with methylandrostenediol (*b*), respectively of young normal female rats (*c*) and born by treated adult rats (*d*). (DPM/cm² = disintegration per minut and 1 cm²) (Hc = hydrocortisone, C = corticosterone, Col. = cholesterol, S = start, F = front.)

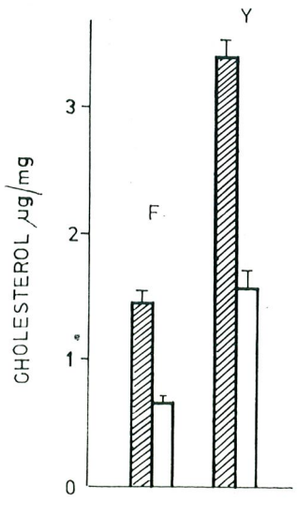

Fig. 3. — Concentration of free cholesterol (µg per mg wet tissue) in adrenals of normal adult rats (F ▨) and treated with methylandrostenediol (F □) respectively in adrenals of young normal female rats (Y ▨) and born by treated adult rats (Y □).

REFERENCES

1. Abraham A. D., *Mecanismul de acțiune al hormonilor steroizi*. Ed. Acad. București, 1975.
2. Baillie A. H., Ferguson M. M., Hart D., *Developments in Steroid Histochemistry*, Academic Press, London, 1966.
3. Bernauer W., Schmidt L., 1963, Arzneimittel-Forschung, **13**, 1075—1077.
4. Hasan S. H., 1976, Pharmac. Ther., **1**, 213—240.
5. Howell S. L., Whitfield M., 1972, J. Histochem. Cytochem., **20**, 873—875.
6. Korenchevschy V. S., Paris S. K., 1950, J. Gerontol, **5**, 120—127.
7. Krüskemper H. L., *Anabole Steroide*, E. Thieme, Stuttgart, 1963.
8. Neher R., *Steroid Chromatography*, Elsevier, Amsterdam — London — New York, 1964.
9. Roy S., Mahesh U. B., 1964, Endocrinology, **74**, 187—192.
10. Saffran M., Vogt M., 1960, J. Physiol., **151**, 123—130.
11. Selye, H., *Hormones and Resistance*, Springer, Berlin— Heidelberg — New York, 1971.
12. Sayers G., Beall R. J., Seelig S., in *Biochemistry of Hormones*, vol. 8, A. V. Rickenberg, Butterworth, London, University Park Press, Baltimore, 1974.

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BIOCHEMICAL CHANGES IN WHITE RATS SUBJECTED TO SUBLETHAL IRRADIATION

BY

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Irradiation of white rats with a single dose of 65 rads of gamma radiation caused some changes, at 2,4 and 9 days after the irradiation. The amount of RNA is decreased post-irradiation but DNA increased in liver and thymus. The nitrogen of the free aminoacids of the liver and thymus showed raised levels 2 and 4 days after irradiation. Nine days following the levels decreased in both organs as well as in the plasma. GPT activity increased in liver and thymus after two days. In the liver and plasma this activity increased after 9 days and in the thymus after 4 days. GOT activity in the liver, thymus and plasma showed a decrease 2 days after irradiation. It remained decreased in liver and increased in the plasma after 9 days.

Irradiation of the animal organism with small doses of gamma rays (2.5—0.6 R) may either stimulate or inhibit some cellular processes [21]. When larger doses of ionizing rays are applied (of the order of 10 R) the changes occurring at the cellular level are not characterized by the structural disorganization that occurs with lethal doses [24].

We undertook the present study to follow the changes induced at different intervals after irradiation with sublethal doses. Some metabolic parameters which are very sensitive to irradiation (the so-called "biological dosimeters"), such as serum transaminase activity, which are involved in protein biosynthesis, were determined.

MATERIALS AND METHODS

Male, white rats, weighing 130—160 g were used. Thirtytwo animals were divided into the following groups :

— control (8), non-irradiated group (C),

— irradiated group (24), exposed to a source of gamma rays (^{60}Co) using the installation THERATRON -80. A single dose of 65 rad for 30 sec. was applied. Groups of 8 animals were sacrified at 2, 4 and 9 days after irradiation, being marked here as subgroup I, II and respectively III.

The animals were maintained on a Larsen diet with water *ad libitum* for the whole period of experiment. After decapitation, samples from liver, thymus and blood (on heparin) were collected. The content on nucleic acids (RNA and DNA [26], total protein [16] and free aminoacid nitrogen [22] were assayed in liver and thymus. In the same organs the activity of two transaminases (glutamate-pyruvate and glutamate-aspartate aminotransferase) were also determined [10].

The results are expressed on the basis of the weight of tissue. In blood plasma, the free aminoacid nitrogen and activity of both transaminases were determined using the same procedures as for liver and thymus.

The data were statistically treated according to the Student "t" test ; the Chauvenet criterion was also applied to exclude highly aberrant values.

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RESULTS

The mean values, standard error and statistical significance of the data are recorded in table 1. The percentage variations of data in the irradiated groups as compared to the control are represented in figure 1 to 5.

Table 1

Changes of parameters in the thymus, the liver and plasma of control and irradiated white rats

Parameters	Organ	Control	Irradiated animals		
			2 days	4 days	9 days
RNA mg/g	Thymus	9.7±0.6(8)	5.8±0.2*(8)	7.6±1*(8)	4.6±0.5*(8)
	Liver	6.9±0.3(8)	4.5±2*(8)	4.9±0.2*(5)	4.0±0.1*(8)
DNA mg/g	Thymus	19.0±0.3(7)	26.8±2.2*(8)	18.1±1.3(7)	22.7±1.5*(8)
	Liver	5.3±0.2(8)	7.6±0.6*(8)	6.2±0,3*(5)	7.2±0.9*(6)
Proteins mg/g	Thymus	23.2±1.0(8)	23.8±1.8(7)	26.1±2.8(7)	22.5±0.7(8)
	Liver	38.5±1.0(8)	31.9±1.8(8)	38.8±2.7(8)	27.5±1.8*(8)
Amino nitrogen	Thymus	13.5±0.7(8)	34.5±6.6*(8)	56.3±15.9*(8)	9.6±1.2*(8)
mg/100 g	Liver	14.3±1.6(8)	14.5±3.5(8)	19.1±1.4*(8)	7.8±0.8*(8)
mg/100 ml	Plasma	5.3±0.7(8)	4.1±0.6(8)	7.6±1.3(7)	3.6±0.3(8)
GPT	Thymus	80±14.5(8)	155±26.9*(8)	35±6.3*(8)	70±2.5(7)
µg/mg	Liver	995±143(7)	2257±450*(8)	1136±205(8)	2208±224*(8)
µg/ml	Plasma	7±0.7(8)	7±0.5(8)	6±0.6(7)	8±0.3(7)
GOT	Thymus	149±12.0(8)	118±0.7*(6)	204±50.7(8)	179±20.4(7)
µg/mg	Liver	1335±145(7)	923±43*(7)	1310±162(8)	1016±84*(8)
µg/ml	Plasma	29±1.8(8)	18±0.7*(6)	24±1.1(7)	34±1.4*(8)
Weight mg	Thymus	224±23.0(8)	164±13.4*(8)	177±11.7(7)	276±23.0(8)

Values represent means ± SEM for number of animals in pharantheses
*—significantly different from control

It is seen from figure 1 A that the RNA content is significantly decreased in both liver and thymus during the whole period after irradiation. The DNA content behaved in an opposite manner (Fig. 1 B).

Total tissue proteins did not change significantly, except for the liver, in the group sacrificed 9 days after irradiation, when there was a significant decrease (Fig. 2).

Liver free aminoacid nitrogen was elevated at 4 days after irradiation and diminished at 9 days (Fig. 3). In the thymus, its content was increased at 2 and 4 days and decreased at 9 days (Fig. 3). A significant diminution of free aminoacid nitrogen was also observed in blood 9 days after irradiation (Fig. 3).

The activity of GPT in liver was increased at 2 and 9 days. In the thymus it increased only at 2 days and at 4 days it decreased (Fig. 4). The activity of GOT in liver and thymus fell significantly 2 days after irradiation. In liver, it did not return to normal (Fig. 5). In blood plasma

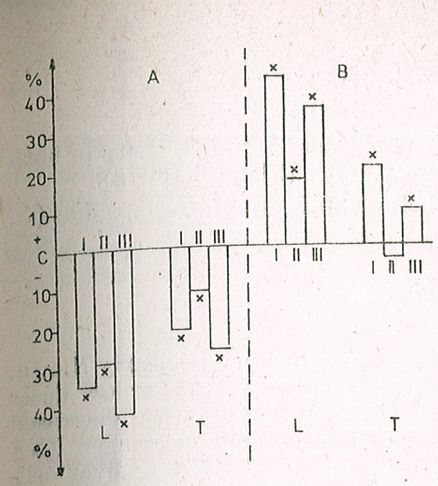

Fig. 1.— The percentage variation of the content of RNA (A) and DNA (B) in the liver (L) and thymus (T), as compared to the controls. I-irradiated group, sacrificed 2 days after irradiation; II-irradiated group, sacrificed 4 days after irradiation; III-irradiated group, sacrificed 9 days after irradiation; ×-statistically significant values.

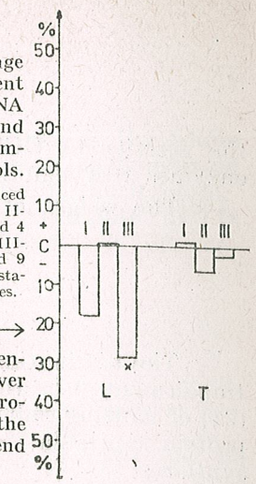

Fig. 2. — The percentage variation of liver (L) and thymus (T) protein content against the control values (Legend as at Fig. 1).

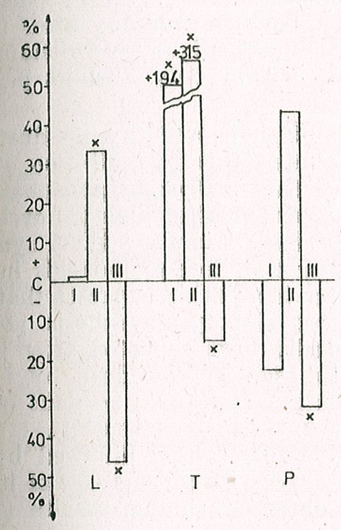

Fig. 3. — The percentage variation of free aminoacids in liver (L), thymus (T) and plasma (P) in comparison to the controls. (Legend as at Fig. 1).

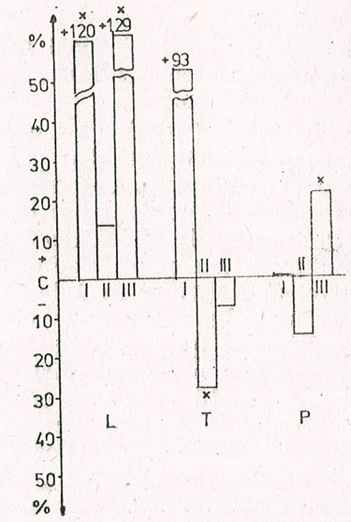

Fig. 4. — Variation of GPT activity in liver (L), thymus (T) and plasma (P), as percentage against the control values. (Legend as at Fig. 1).

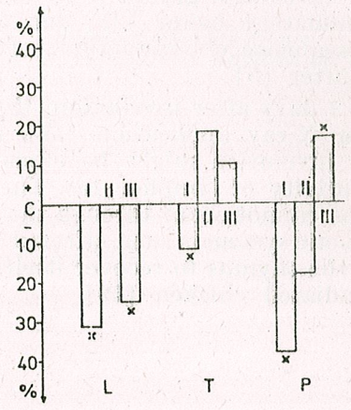

Fig. 5. — The percentage variation of liver (L), thymus (T) and plasma (P) GOT activity against the control values. (Legend as at Fig. 1).

the activity of GOT was diminished at 2 days; the activity of both enzymes was significantly decreased at 9 days (Figs. 4 and 5).

The weight of thymus was decreased at 2 days after irradiation.

DISCUSSION

The diminution of the amount of protein in liver is clearly shown. Impairment of protein synthesis in the liver after high (3,25) or low doses (24) of ionizing radiation is well known. The main cause of the decreased protein synthesis seems to be alteration of nuclear and messenger RNA synthesis [3]. It is known that the total content of RNA in liver cells is linked to the concentration of free aminoacids [28]. The decreased concentration of free aminoacids in liver on the 9th day after irradiation associated with a diminished RNA content might be the main cause of an impaired protein synthesis, even within only a short period of time after irradiation.

The decrease of total RNA content in liver and thymus during the whole post-irradiation period might be due either to inhibition of RNA polymerase (11,18) or to a decrease of the nucleozide phosphate pool [24]. A rise of DNA content has been reported after high (4) or low (8) doses of irradiation. It seems that these doses stimulate DNA synthesis.

The diminution of the amino nitrogen of free aminoacids in blood plasma, liver and thymus at 9 days after irradiation suggests that these aminoacids may be extensivley used for glycogen synthesis [14]. An increased glycogen synthesis has repeatedly been reported after irradiation of the whole organism with both high or low doses of ionizing radiation [2, 4, 6, 15]. Such an effect might appear on account of compounds occurring in some other radiation sensitive tissues [15].

The application of small doses of gamma rays is followed by a decrease of liver GOT activity [20], associated with an increase of its activity in blood plasma. Other authors obtained similar results [1, 19]. This confirms that ionizing radiations affect both the enzymatic activity [13] and the cell membrane permeability [8]; as a consequence, the enzymes are released from the liver cell into the blood [23].

In our experimental conditions, at 2 days after irradiation the activity of GPT had a higher value in both thymus and liver as compared to the control group. It is possible that the radiation energy acts as a stimulatory factor [21] due to a new status created in the cell as a consequence of interaction between the ionizing radiation and the living matter [5].

At 2 days after irradiation, thymus involution occurs, as observed also after X-ray application [17], followed by recovery after 3 days. Such an involution might be explained on the basis of a high radiation sensitivity of lymphocytes. The recovery of the thymus under our conditions cannot occur through the bone marrow cells, since the whole haemopoietic system is such affected by gamma rays [7]. It took some time for the thymus to recover itself. This was also observed for thymus in X-irradiated chicken [12].

The changes here reported, induced by irradiation of the whole organism, might also be due to some indirect effects of such ionizing radiations upon regulatory systems. Thus, stimulation of the hypothalamus-hypophysis-adrenal cortex system [9, 27] can stimulate ACTH and glucocorticosteroid secretion, with their consequences on protein-metabolism.

REFERENCES

1. Almonte T. J., Barnes J. H., Bautista S. C., 1965, Nucl. Sci. Abs. **19**, 36012.
2. Agarwal S. K., Mehrotro R. M. L., 1965, Nucl. Sci. Abs., **19**, 33844.
3. Ariyama K., Fausto N., Tamvakopoulos E. and Langker J. L., 1970, Rad. Res., **42**, 528–538.
4. Bacq Z. M. and Alexander P., *Fundamentals of radiobiology*, Pergamon Press, Oxford — London — New York — Paris, 1963.
5. Benetato G., Capalna S., Ghizari E. and Stefan M., 1970, St. Cerc. Fiziol., **15**, 291–295.
6. Berndt J., 1969, Nucl. Sci. Abs., **23**, 26701.
7. Blomgren H., 1969, Exptl. Cell. Res., **58**, 353–364.
8. Butculescu I., *Cercetări biochimice asupra reglării hepatice*, Cluj, Teză, 1972.
9. Dedov V. I., 1972, Nucl. Sci. Abs., **26**, 7088.
10. Fauvert R., *Technique moderne de laboratoire*, Paris, Ed. III, 1961.
11. Fox K. E. and Gabourel I. D. 1972, Endocrin., **90**, 1388–1390.
12. Giurgea R., Manciulea S. and Ilyes I., 1975, Acta Vet. Hung., **25**, 205–209.
13. Heilmeyer L., *Fiziologie Specială*, Ed. Med., Bucureşti. 1968.
14. Kay R. E., Harris D. C. and Entenman C., 1956, Amer. J. Physiol., **186**, 175–179.
15. Kendysh I. N., Fedotov V. P., Moroz B. B. and Dubrovin A. D., 1969, Nucl. Sci. Abs., **23**, 24518.
16. Kovách A. A., *A kisérletes orvostudomány vizsgáló módszerei*, Acad. Kiado. **II**, Budapest, 1958.
17. Milcu S. and Lungu A., *Hormonii şi viaţa*, Ed. Ştiinţ. Bucureşti, 1965.
18. Nakagawa S. and White A., 1966, Proc. Nat. Acad. Sci. USA, **55**, 900–904.
19. Nomura S., 1969, Nucl. Sci. Abs., **23**, 41443.
20. Pora E. A. and Manciulea S., 1969, St. Cerc. Biol., **21**, 327–332.
21. Pora E. A., Manciulea S. et Rusdea-Suteu D., 1968, Rev. Roum. Biol., **13**, 193–195.
22. Rac I., Casop., 1959, Likar. Cesk. **98**, 120–123.
23. Ramos R. E., 1969, Nucl. Sci. Abs., **23**, 50354.
24. Raţiu H., *Acţiunea dozelor mici de radiaţii Röntgen asupra acizilor nucleici din sistemul nervos central*, Cluj, Teză, 1971.
25. Stantagati G., Slaviero G. E., Cultrera G., Bosco G., 1965, Arch. Sci. Med., **119**, 48–56.
26. Spirin A. S., 1958, Biochimia, **23**, 656–662.
27. Törö T., Bácsy E., Ökrös I., Vadász G. and Rappay G., 1968, Acta Medica Acad. Sci. Hung., **25**, 355–360.
28. Wannemacher R. W., 1971, Biochem. J., London, **124**, 385–392.

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EFFECTS OF FASTING AND GLUCOSE ON THE ELECTRICAL ACTIVITY OF THE HYPOTHALAMUS IN CHICKENS

BY

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Electrical activities of the chicken lateral (LH) and posteromedial hypothalamus (HPM) were recorded postprandially, and at 24, 48, 72 and 96 hours following suppression of food intake. A low amplitude and a relatively fast activity appeared in the LH of fasted chickens. Intracrop glucose administration extinguished this arousal. Contrary to these effects, prolonged food deprivation induced a slow activity of high amplitude in HPM, but they disappeared on intracrop glucose administration.

The electrical activity of the ventromedial and lateral hypothalamus appears to be related to the feeding state of the animals. Changes in both the electroencephalogram (EEG) and the unit activity from these hypotahlamic nuclei during fasting or following glucose administration in various mammalian species have been reported by a number of workers [1—3], [6—9], [12], [14].

Little is known about the correlation between the electrical changes in the hypothalamus of birds and their feeding state [4].

In the present paper we report the effects of prolonged food deprivation and intracrop glucose administration, on the electrical activity of the hypothalamus in chickens.

MATERIAL AND METHOD

Six Leghron hens weighing 1,300—1,800 kg were used in the experiment. Under pentobarbital anaesthesia (30 mg/kg b. w.), the birds were placed in a stereotaxis apparatus and two pairs of stainless steel electrodes were implanted, one in the lateral hypothalamus (LH), and the other in the posteromedial hypothalamus (HPM), on either side of the 3rd ventricle, using the coordinates given in the Van Tienhoven and Juhasz atlas [13]

The electrodes were insulated except for the last 0.5 mm at the tip, and had conically sharpened tips of 10 μ in diameter.

After recovery from surgery, the birds were submitted to 96 hours of food deprivation and bipolar recordings of electrical activity from hypothalamus were made postprandial at 24, 48, 72 and 96 hours following suppression of food. The profil of EEG was also studied following intracrop administration of glucose (6 ml of 5% solution at 20 °C, by means of a polyethylene cannule) in 96 hours food deprived birds.

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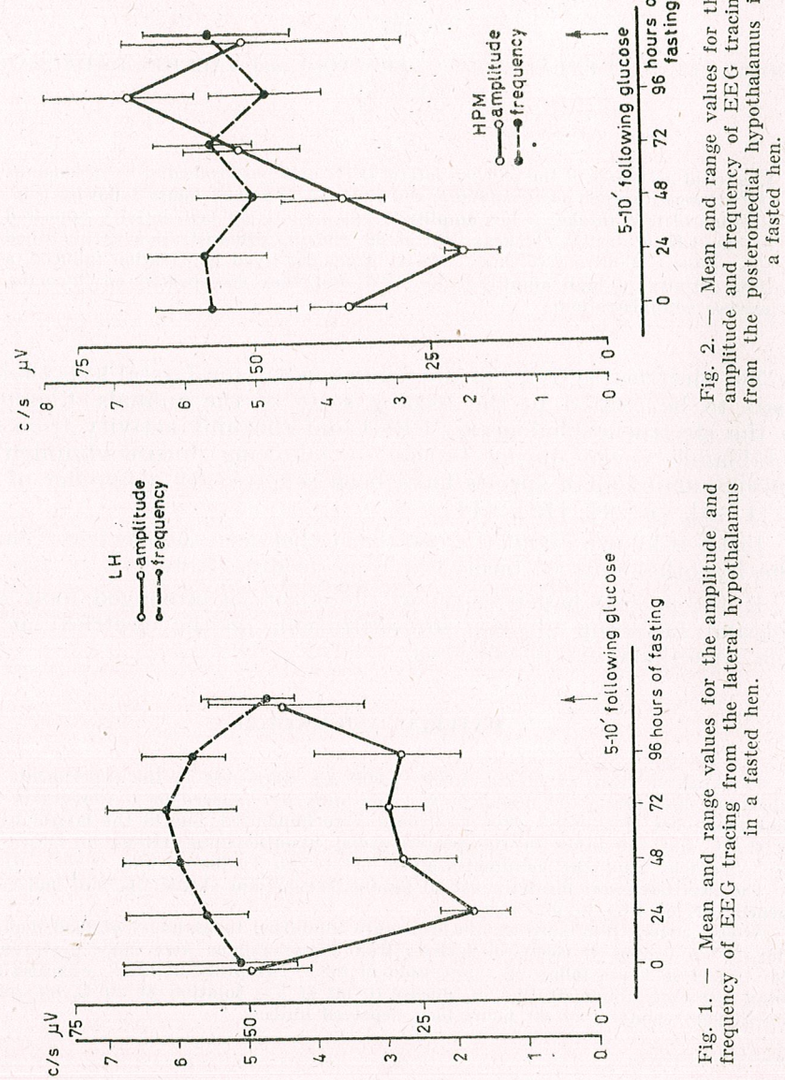

Fig. 2. — Mean and range values for the amplitude and frequency of EEG tracing from the posteromedial hypothalamus in a fasted hen.

Fig. 1. — Mean and range values for the amplitude and frequency of EEG tracing from the lateral hypothalamus in a fasted hen.

The chickens were tested in an insulated experimental box with one-way glass observation window. Recording of the EEG tracings was made with a four-channel electroencephalograph "Officine Galileo". At the end of the experiment all the birds involved were intracarotidally perfused with 0.9% saline solution followed by 10% Formalin. The brains were then embedded in paraffin, sectioned in 10 μ slices and stained with hemalaun Mayer and erythrozin-orange G.

Examination of the brain sections indicated that the electrode tips were located in the lateral and posteromedial (ventromedial) regions of the hypothalamus in four of the six tested chickens. These chickens also responded by a more or less marked hyperphagia to the stimulation of the lateral hypothalamus, even when they were previously well fed.

The amplitude of EEG waves was evaluated by using the planimetric method of Racotta [10] on 15—20 EEG sectors of 5 seconds each. The frequency was also established by measurement of the complete wave on the planimetred EEG traces.

The values obtained for the amplitude and frequency of the various sectors examined, were used to compute the "mean" amplitude and the "mean" frequency as well as the standard error of the mean. Starting from these values, we then tried to establish for each hen the "significance" of the differences found between the mean values of the amplitude and frequency of the EEG waves recorded postprandial, at different hours from food suppression and after intracrop glucose administration by applying the Student "t" test.

RESULTS

Figures 2 and 3 illustrate the mean and range values for amplitude and frequency of EEG tracings recorded from the lateral and posteromedial hypothalamus in one of the birds submitted to the above mentioned treatment and which postprandially showed an exagerated hyperphagia after stimulation with the pair of electrodes planted in the lateral hypothalamic area. An analysis of these values revealed significant differences ($p < 0.01$) among the changes in EEG from these regions under the influence of prolonged fast and intracrop glucose.

Thus, in the lateral hypothalamus the satiation state was associated with a relative high amplitude, slow waves, and fasting, with low voltage, faster activity. Intracrop glucose administration after 96 hours of food deprivation was shortly followed by the appearance of higher amplitude and lower frequency patterns.

In the posteromedial hypothalamus, fasting evoked only on the first day a low voltage, relative fast EEG activity. As fast was prolonged, the waves increased progressively in amplitude and their frequency tended to decrease. Glucose administration following 96 hours of food deprivation was accompanied by a reduction in the amplitude and a slight rise in the frequency of the waves.

Similar tendencies had the changes in the brain electrical activity in all fasted chickens with a correct placement of the electrodes in the hypothalamus.

DISCUSSION

The results of this study show that both fasting and glucose administration affect the electrical activity of the lateral and posteromedial hypothalamus in chickens. The EEG changes appear different depending on the nucleus. Thus, prolonged food deprivation seems to induce a persistent arousal only in the electrical activity of the lateral

hypothalamus, as it was demonstrated by the decreasing amplitude and the slightly increasing frequency of the waves recorded from this hypothalamic area. Similar changes were reported by Gasanov in fasted pigeons [4].

Intracrop glucose seems to activate, on the other hand, the EEG patterns from posteromedial nucleus of hypothalamus and extinguish the activating effect of fasting upon the lateral hypothalamus. The fact that the EEG changes differed for different nuclei shows that we do not deal with a nonspecific effect produced by stimulating the crop with introduction of a liquid, which is known to induce EEG arousal in chickens [5]. This rather implies the existence of glucose sensitive neourons within the hypothalamus of the chickens which would suggest a dual mechanism, with a feeding and a satiety center in the nervous control of food intake in these birds. The presence of a delimited satiety center in the ventromedial hypothalamus of chickens also resulted from the experiments of Snapir et al. [11].

Investigations of the direct effects of glucose on individual cells of the lateral and posteromedial hypothalamus would contribute to explain how the cells of these nuclei are activated and what are the reciprocal relations between their activities. Such investigations have been performed until now only on the hypothalamic feeding centers of mammals [2], [7], [8], [9].

REFERENCES

1. Anand B. K., S. Dua B. Singh, 1964, EEG clin. Neurophysiol., **13**, 54–59.
2. Anand, B. K., G. S. Chhina, K. N. Sharma, S. Dua, B. Singh, 1964, Amer. J. Physiol., **207**, 1146–1154.
3. Chhina, G, S., B. K. Anand, B. Singh, P. S. Rao, 1971, Amer. J. Physiol., **221**, 662–667.
4. Gasanov, G. G., V. V. Rubtzova, *K voprosu o neirohimiceskih mehanizmah piscevogo vozbujdenia u golubei v raznie periodi golodania*, Izv. Akad. Nauk. Az. S. S. R., Ser. biol. **3**, 73–81, 1970.
5. Gentle, M. J., A. Richardson, 1972, Br. Poult Sci. **13**, 163–170.
6. Hamburg M. D., 1971, Amer. J. Physiol., **220**, 980–985.
7. Oomura, J., H. Ooyama, T. Yamamoto, F. Naka, 1967, Physiol. Behav., **2**, 97–115.
8. Oomura, J., T. Ono, H. Ooyama, M. J. Wayner, 1969, Nature, **222**, 282–284.
9. Oomura, J., H. Ooyama, M. Sugimori, T. Nakamura, Y. Yamada, 1974, Nature, **247**, 284–286.
10. Racotta, R., 1968, Acta biol. med. germ., **21**, 245–248.
11. Snapir, N., B. Robinzon, M. Godschalk, E. D. Hellek, M. Perek, 1973, Physiol., Behav., **10**, 97–100.
12. Sudakov, K. V., 1965, Fiziol. J. SSSR, **51**, 449–456.
13. Tienhoven, A. Van, L. P. Juhasz, 1962, J. comp. Neurol., **118**, 185–197.
14. Voronin, L. G., L. V. Kaliuznii, I. N. Zaharova, 1965, J. viss. nerv. deiatel., **15**, 364–373.

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THE EFFECT OF THYMOSTERIN ON ROSETTE FORMING CELLS (RFC) IN NEONATAL THYMECTOMIZED RATS

BY

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The neonatal thymectomy of Wistar rats reduces the number of rosette forming cells (RFC) with 91.5%. After a treatment during 13 weeks with thymosterin (0.2 ml/2 days) appears a partial restauration in the number of RFC, as compared to the thymectomized group.

Since the perinatal thymectomy induces an inevitable "wasting syndrome" [4], it seems that the thymus is an essential organ for the development of immunological competence. Reconstitution of immunological responsiveness with thymus grafts included in "Millipore" chambers or gland factors leads to a hormonal mechanism in the development and differentiation of active T lymphocytes involved in cell mediated immunity [2, 3, 5, 7].

In the present experiments the effects of thymosterin of Potop and Milcu [6], with antitumour activity, have been studied on RFC of neonatally thymectomized rats.

Wistar rats were operated within 18 hours after birth and divided in the following three groups (Table 1):

(1) sham operated controls;

(2) thymectomized;

(3) thymectomized, injected with thymosterin (0.10 ml/30 g b. w./2 days).

Table 1

Effect of Thymosterin on RFC of neonatally thymectomized rats

Groups	RFC/10.000 spleen cells		
	Mean ±S.E.	Modif.	p
1. Controls	177.7±20.7 (n=7)	—	—
2. Thymectomized	15.3±1.5 (n=6)	−91.5	0.001
3. Thymectomized + Thymosterin	28.5±5.1	+85.5*	0.01

n=number of experiments;

*=differences between the groups 2—3.

After 13 weeks of experiments, all the animals were injected with sheep red blood cells (SRBC) and 5 days later the RFC were tested according to Bach [1].

The neonatal thymectomy induced a decrease of RFC by 91%, $p < 0.001$ showing the immunological deficit, which has been demonstrated by other tests too [2]. The treatment with Thymosterin restored partly the cell mediated immunity of thymectomized animal, the difference between groups 2—3 (+ 85%) are statistically significant, even though the RFC values were lower than in controls. These data are in agreement with Goldstein's hypothesis, according to which the site of action of thymic hormones in lymphocyte differentiation is localized in the gland itself in whose absence, the immunitary restitution is not complete [3].

REFERENCES

1. Bach J. F., 1972, La téchnique des rosettes, signification immunologique et application clinique. Les Cahiers de Médicine, **13**, 907—914.
2. Escande J. P., Cambier J., 1970, Le Thymus. Rev. Prat., **20**, 3717—3726.
3. Goldstein G., 1972, Les hormones du Thymus. Triangle, **11**, 7—14.
4. Miller J. F. A. D., Marshall A. H. E., White R. G., 1962, The immunological significance of the thymus. Adv. Immunol., **2**, 111—162.
5. Müller H. C., Schmiege S. K., Rule A., 1973, Production of functional T cells after treatment of bone marrow with thymic factor. J. Immunol., **111**, 1005—1012.
6. Potop I., Milcu St. M., 1973, *Biologic acitvity and structure of thymic lipids and Thymosterin*, in : *Thymic Hormones*, ed. by T. D. Lukey, p. 205—271, Ed. University Park Press, Baltimore — London — Tokyo.
7. Raff M. C., 1973. T and B lymphocytes and immune responses. Nature (G. B.), **242**, 19—23.

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REAKTIONEN DES THYMUS UND DER BURSA FABRICII NACH BEHANDLUNG MIT DEM ZYTOSTATIKUM I. O. B.-82

VON

RODICA GIURGEA, VIRGIL TOMA und MAGDA KOSZTA

The i. p. injection of the cytostatic I. O. B.-82, in a single dose of 1 mg/kg b. w. in 5-day-old chicken lead to modifications in the metabolism nucleic acids and global proteins in thymus and bursa Fabricii. These may be attributed to the alkilation of the purinic and pyrimidinic bases in DNA and RNA giving denaturated produces which are immunological inactive. After 8 days onward from the injection there intervenes a pronounced depletion in ascorbic acid in the adrenals which denotes a hypersecretion of glucocorticosteroids leading to a thymobursal involution.

Verabreichen von Zytostatika vervollkommen chirurgische und radiologische Behandlungen von bösartigen Tumoren. So ein Zytostatikum das sich durch eine spezifisch antitumorale Wirkung und eine herabgesetzte Toxizität auszeichnet, wurde vom Bukarester Onkologischen Institut hergestellt ; I. O. B.-82 benannt, und ist eine 3N, N'bis (2 chlorethyl)-amino 4 methyl Benzoesäure [1] [2]. Es ruft humorale und zelluläre Immunosupression hervor wie auch histopathologische Veränderungen in Thymus, Milz und Lymphganglien [3].

Da Thymus und Bursa Fabricii zentrale Lymphorgane der Vögel sind, haben wir die Auswirkungen von I. O. B.-82 auf die Konzentration der Nukleinsäuren in diesen Organen verfolgt, vor allem weil diese Bestandteile eine Hauptrolle in den immunologischen Reaktionen spielen [4]. Gleichzeitig wurde auch der Askorbinsäuregehalt der Nebennieren dosiert, der als Streßindikator des Giftigkeitsgrades des Zytostatikums dient.

MATERIAL UND METHODEN

Kücken, tetralineale Hybride der Rasse Studler-Cornish, 5 Tage alt, in Laborbedingungen, dienten als Versuchsmaterial.

I. O. B.-82-wurde 1 mg/Kg Körpermasse in Polyethylenglykol und einer Stabilisationslösung intraperitoneal einmalig injiziert ; die Kontrolle bekam das gleiche Flüssigkeitsvolumen ohne dem Zytostatikum.

Behandelte und Kontrolltiere wurden nach 8 Studen, 1, 4, 8, 14 und 21 Tagen durch Köpfen getötet. Thymus, Bursa und Nebennieren wurden sofort entnommen und folgenden quantitativen Analysen unterzogen :

— DNA und RNA nach der spektrophotometrischen Methode Spirin [12] ;
— Gesamtproteine (GP), nach Gornall u. Mitrab. [6] ;
— Askorbinsäuregehalt nach Klimov [8].

Die Ergebnisse wurden statistisch nach Student geprüft, aberrante Werte nach dem Chauvenet-Kriterium ausgeschlossen.

Tabelle

Eiweissgehalt von Thymus und Bursa Fabricii

Indikator		Kontrolle		8 Stunden		1 Tag		4 Targe	
		Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus
Gesamt-proteine (µg/mg)	Mittel	136,2	195,8	180,0	178,4	231,5	313,2	196,6	302,3
	ES±	4,4	7,8	6,1	9,6	12,7	12,8	8,5	30,1
	n	8	8	8	8	8	8	8	8
	p	—	—	<0,01	—	<0,001	<0,001	<0,001	<0,01
RNS (mg/g)	Mittel	6,3	5,9	7,1	7,6	5,0	6,2	6,2	5,4
	ES±	0,2	0,5	0,2	0,3	0,2	0,3	0,3	0,3
	n	8	8	8	8	8	8	8	8
	p	—	—	<0,05	<0,05	<0,001	—	—	—
DNS (mg/g)	Mittel	8,4	12,9	9,0	20,0	9,2	12,4	10,9	13,0
	ES±	1,6	0,89	0,50	0,2	0,6	0,4	1,7	1,2
	n	8	8	8	8	8	8	8	8
	p	—	—	—	<0,001	—	—	—	—
Gewicht Organ (mg)	Mittel	61,7	71,1	63,8	91,8	43,8	58,7	52,8	45,0
	ES±	7,4	7,7	5,6	10,6	6,0	8,6	10,1	8,3
	n	8	8	8	8	8	8	8	8
	p	—	—	—	—	<0,05	—	—	<0,05

Zeichenerklärung: ES± = Eronata Standard; n = Anzahl der untersuchten Tiere; p = statistische Signifikanz

ERGEBNISSE UND DISKUSSIONEN

Nach Tabelle 1 und Abb. 1, ist augenfällig, daß der Thymus gegenüber der Bursa unterschiedliche Reaktionen aufweist. Die quantitativen Unterschiede sind auf die induzierten immunitären Zell-und Humoralprozesse der Lymphozyten T und B zurückzuführen [7] [9].

Die immunosuppressive Wirkung von I. O. B.-82 kann der Beschädigung des Nukleinsäuremetabolismus zugeschrieben werden. Obwohl in den ersten 8 Stunden die Nukleinsäuremenge im Thymus ansteigt, betrachten wir das nicht als eine immunitäre Stimmulierung. Wie bekannt, alkilieren solche Zytostatika die NH der Purin und Pyrimidinbasen was zu Entartungen in den Synthesen der Nukleoproteide und Proteine führt [10]. Abnorme Beziehungen zwischen den Nukelinsäuren können mit der progressiven Inhibition der RNA-Synthese zusammenhängen. Die Erhöhung der Gesamtproteine in Thymus und Bursa kann im Zusammenhang mit der durch Alkilierung verstimmten Proteinsynthese erklärt werden. Die Folgen der von I. O. B.-82 hervorgerufenen Störungen in den Zentralkomponenten des Immunapparates wurden von Dobre [3], erklärt. Dieser Autor beschreibt eine beträchtliche Abnahme der Leukozytenaktivität und der peritonealen Makrophagen, den Abstoß der Allografen, die Synthese von Antikörpern und eine verspätete Hypersensibilisierung.

1

bei Kücken nach akuter Behandlung mit I.O.B.-82

Kontrolle		8 Tage		Kontrolle		14 Tage		Kontrolle		21 Tage	
Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus
119,3	209,2	173,1	273,5	142,4	180,0	160,3	248,0	176,1	193,7	168,4	217,8
4,0	7,8	6,0	13,3	3,9	4,1	4,4	20,3	4,8	19,5	3,5	10,7
8	8	8	8	8	8	8	8	8	8	8	8
—	—	<0,01	<0,001	—	—	—	<0,01	—	—	—	—
7,1	5,8	2,1	2,2	7,1	7,4	1,3	1,8	8,8	7,8	5,2	5,9
0,3	0,4	0,1	0,1	0,1	0,2	0,06	0,1	0,7	0,4	0,2	0,2
8	8	8	8	8	8	8	8	8	8	8	8
—	—	<0,001	—	—	—	<0,001	<0,01	—	—	<0,05	<0,05
8,4	11,3	10,9	13,2	11,9	16,5	12,7	16,1	9,5	15,4	7,3	17,6
0,5	1,04	0,5	0,3	0,6	0,3	0,6	0,9	0,6	0,6	0,5	0,3
8	8	8	8	8	8	8	8	8	8	8	8
—	—	—	—	—	—	—	—	—	—	—	—
95,6	71,6	76,1	84,6	278,1	394,3	140,0	125,4	389,6	539,5	576,5	691,7
11,5	14,2	11,8	19,7	28,6	20,2	31,8	26,5	44,4	73,5	37,4	27,6
8	8	8	8	8	8	8	8	8	8	8	8
—	—	—	—	—	—	<0,01	<0,001	—	—	<0,01	<0,0 5

Eine Erscheinung die praktisches Interesse in der Behandlung mit Zytostatika hat, ist die Variation des Askorbinsäuregehaltes aus den Nebennieren. In den ersten 4 Tagen nach der Injizierung des Medikaments wächst der Askorbinsäuregehalt signifikant an, was auf eine Stagnation der Glukocortikoidsynthese hinweist. Anderseits, nach dem achten Tag beginnt eine Verminderung des Askorbinsäuregehaltes, was mit einer Hypersekretion der Nebennierenrinde zusammengeht [11]. Dies ist mit einer gleichzeitigen Involution von Thymus und Bursa bewiesen [5] [13]. Die Bedeutung und die Mechanismen dieser biphasischen Reaktionen sind noch schwer zu erklären. Wahrscheinlich hat das Zytostatikum anfangs einen Hemmungseffekt in den energetischen Prozessen der Synthese der Glukokortikoidhormone und maskiert eine Zeit lang seine eigene Streßwirkung.

Als Schlußfolgerung, ruft die Injizierung des Zytostatikums I.O.B.-82, 1 mg/Kg Körpergewicht bei 5 tägigen Kücken, thymo-bursale Reaktionen hervor die der Alkilierung der Purin und Pyrimidinbasen der Nukleinsäuren zuzuschreiben sind.

Als eine Folge dieser biochemischen Reaktionen stellen sich zytostatische und immunosuppressive Wirkungen der Medikaments ein.

Vom 8-ten Tage an tritt eine signifikante Abnahme der Askorbinsäure aus den Nebennieren hervor; die Hypersekretion der Glukokortikoiden ruft ihrerseits eine thymo-bursale Involution hervor.

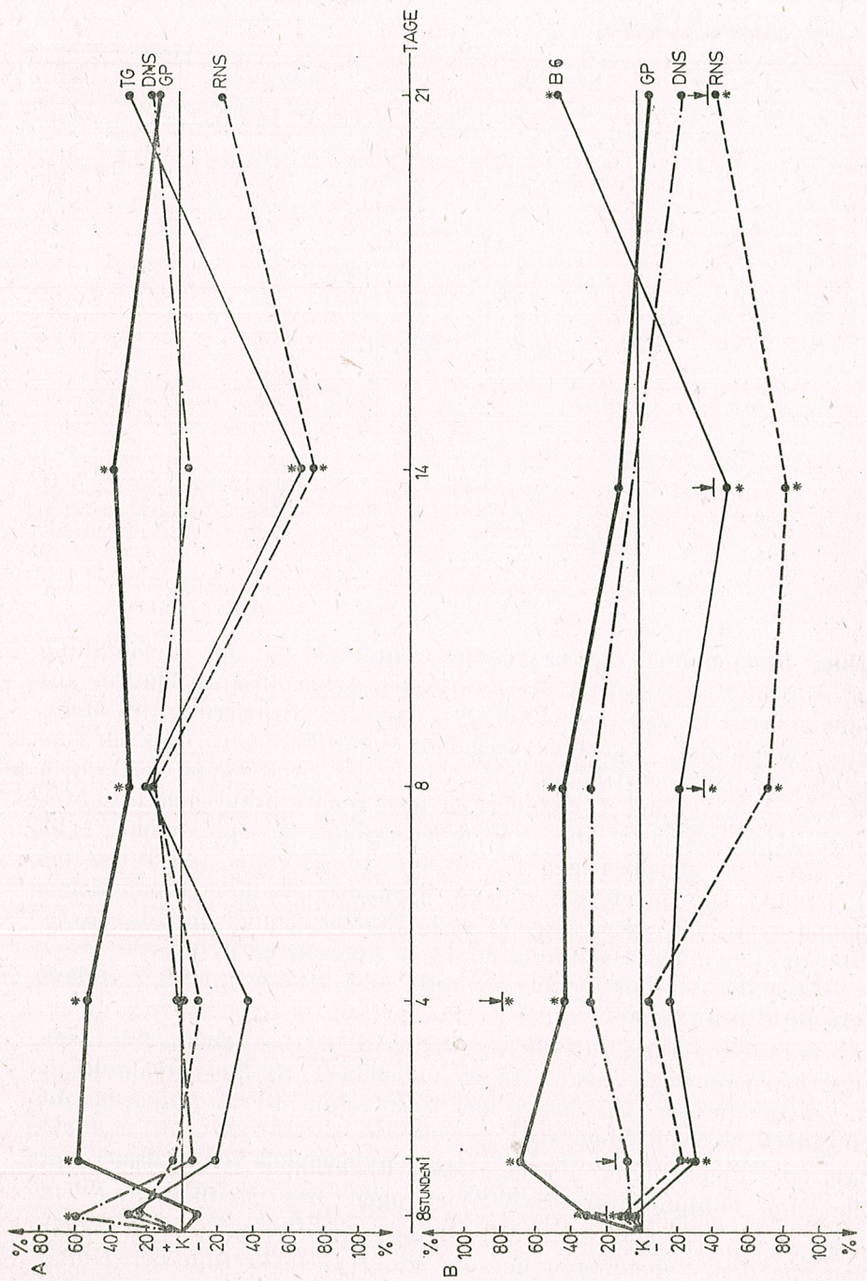

Abb. 1.— Die Dynamik der Nukleinsäuren (RNS und DNS), der Gesamtproteine (GP) und Organ-Gewicht (Thymus-Gewicht, TG, und Bursa-Gewicht, BG), des Thymus A) und Bursa Fabricii B) nach Verabfolgung von I. O. B.-82. Der Pfeil zeigt die Variationen der Askorbinsäure in den Nebennieren. + Signifikante Werte.

LITERATUR

1. Dobre V., Maltezeanu Gr., 1968, Oncol. Radiol., **7**, 237.
2. Dobre V., Maltezeanu Gr., 1970, Canc. Chem. Rep., **54**, 319.
3. Dobre V., Acţiunea citostaticelor în răspunsul imunobiologic, 1975, Teză de doctorat, Universitatea Babeş-Bolyai, Cluj-Napoca.
4. Friedman H., 1973, *RNA in immune response*, Ed. New York Acad. Science, New York.
5. Giurgea R., Toma V., 1975, Zbl. Veti Med., A, **22**, 485.
6. Gornall A. G., Bardawill, G. J., David M. M., 1949, J. Biol. Chem., **177**, 751.
7. Haurowitz F., 1973, Ann. New York Acad. Sci., **207**, 8.
8. Klimov A. N., 1957, Bioch. fotometria, **1**, 311.
9. Moticka E. J., 1975, Amer. Zool., **15**, 135.
10. Păun R., Ursea N., Luca N., Coculescu M., Luca R., 1972, *Terapia imunosupresivă*, Ed. medicală, Bucureşti.
11. Pokrovsky A. A., Korovnikov K. A., 1976, 10th Inst. Congr. Biochem. Abstr. Nr. 07—5—176, 46.
12. Spirin A. S., 1958, Biochimia, **23**, 656.
13. Toma V., Giurgea R., 1974, Zbl. Vet. Med. A, **21**, 506.

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A COMPARATIVE STUDY BEARING UPON THE REST-METABOLISM RATE (RMR) VALUES IN SPECIES *CITELLUS CITELLUS* (L.), *MESOCRICETUS NEWTONI* (NEHR.) AND *MESOCRICETUS AURATUS* (WATERH.) DURING THE POSTNATAL DEVELOPMENT

BY

DOMNICA TÂCU

Over the first three life weeks, RMR shows but a slight increase in species *C. citellus*, maximal values and top fluctuations being recorded in the second month. Thus, the fourth-fifth life week, when the eye opening occurs, corresponds to the highest energy metabolism value to be found in this species (16.5±2.1 kcal /kg/h).

In species *M. newtoni* and *M. auratus*, a rapid RMR evolution is to be recorded in the first life month, top values being also found in the moment eyes open, i. e. at two weeks' age (25.8±4.6 kcal/kg/h and 25.1 kcal/kg/h, respectively). In both species, RMR reaches the adult's values at 10—13 weeks of age (10.7 and 9.8 kcal/kg/h, respectively).

The researches bearing upon the energy metabolism changes in ontogenesis are important from both the theroretical and practical viewpoints. The knowledge of the age peculiarities appears compulsory when the energy flow rate is to be established in the rodent populations, the damages caused by them to the agricultural cultures are evaluated, and indirectly when the optimal conditions for the development of various animal species useful to man have to be figured out.

Species *Citellus citellus* and *Mesocricetus newtoni* are frequently met with in the fallow and cultivated soils of Dobrogea Romania). The present paper analyzes some aspects related to the value of the rest-metabolism rate values (RMR) in ontogenesis, as well as to the comparative study of these values in species *Citellus citellus*, *Mesocricetus newtoni* and *Mesocricetus auratus*.

MATERIAL AND METHOD

Animals belonging to species *Citellus citellus* and *Mesocricetus newtoni* were captured from the fallow soil of the Valu-lui-Traian commune, Dobrogea, southern part of Romania.

The females of both species brought forth in the laboratory. The value of the rest-metabolism rate was followed up in the newborn animals of both species by starting with the age of two days and up to the adult stage. Once in three days, the newborn individuals were weighed and submitted to RMR determinations. Between the experiments, the youngs were allowed to suck from their mothers. The milking females were fed with maize and lucerne and were given water *ad libitum*. The little ones were also let to the same food.

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For the sake of data comparing, the evolution of RMR was analyzed also in the adults. To the same end, we give further on the table of RMR evolution in newborn individuals of *Mesocricetus auratus* as already published [5].

The value of the rest-metabolism rate was assayed by means of the O_2 consumption and CO_2 production which were analyzed with a Li_3 interferometer. The experiments were carried out at a 20 °C temperature and the results expressed in kcal/kg/h.

RESULTS

As shown in table 1 and in figure 1, the *Citellus citellus* little ones opened their eyes at four-five weeks' age. Between this age and that of seven weeks they stopped being fed by their mothers. The weight gain was

Table 1

RMR in adult *C. citellus* (L.) and *M. newtoni* (Nehr.) at 20°C±1.

Species	N° of anim.	Body weight (g)	RMR (Kcal/kg/h)
C. citellus	22	174±19.7	10.8±1.8
M. newtoni	6	136±0.0	9.3±1.1

rather slight during the first three weeks, but went on increasing up to the age of 20 weeks, when the weight reached the standing values of the adults. The evolution of RMR was slow in the first three weeks (7—9 kcal/kg/h) (Fig. 2). After this time period, the RMR values rapidly climbed up to the maximal values at five weeks of age (16.5 ±2.1 kcal/kg/h), a moment which corresponds to the eye opening. In species *C. citellus*, higher values of the energy metabolism have been recorded in the second life month, although large fluctuations have been observed, as well. The fact is noteworthy, however, that after 13 weeks'age, the values of the rest-metabolism rate in the *C. citellus* little ones appear rather close to the values recorded in the adults (Table 1, and Fig. 2). Heavy fluctuations are to be observed, however, since species *C. citellus* raises adaptation problems showing poor tolerance for captivity as compared to species *M. newtoni* or *M. auratus* which may easily be domesticated.

The little *M. newtoni* ones opened their eyes at two or three weeks of age, while, after about five weeks from birth, the females stopped suckling them. The weight gain appeared obvious and smooth up to the age of ten weeks when it became constant and equalled the values found in the adults (Table 1 and Fig. 1). The value of RMR showed large oscillations (Fig. 2) and appeared in the first week to be low (7.0±1.6); the maximal level was reached at two weeks' age (25.8±4.6 kcal/kg /h) corresponding to the eye opening.

As compared to the adults, the values were higher and considerable fluctuations were recorded up to the age of five weeks. In the second month, RMR decreased and less variations were observed, the adult values being equaled at 10 weeks (9.8 kcal/kg/h) and constant RMR values recorded after this time period.

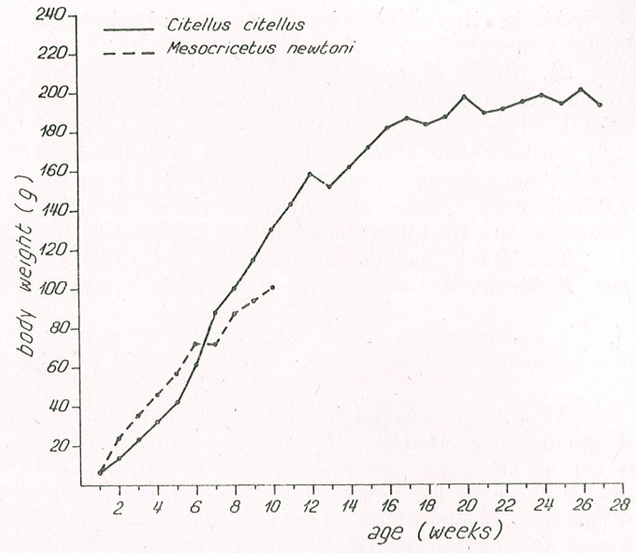

Fig. 1. — Body weight in new-born *Citellus citellus* L. and *Mesocricetus newtoni* Nehr.

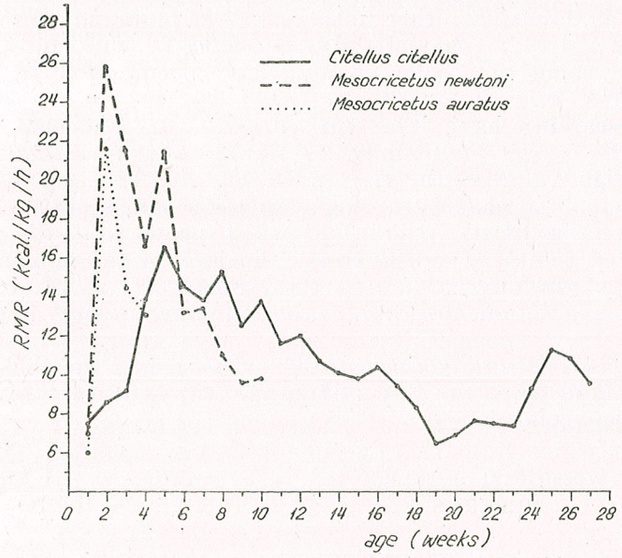

Fig. 2. — RMR in newborn *Citellus citellus* L., *Mesocricetus newtoni* Nehr. and *Mesocricetus auratus* Waterh.

By comparing the two species *(C. citellus* and *M. newtoni)* (Figs. 1 and 2), one may observe the parallel evolution of the weight gain, as well as great resemblances as far as RMR is concerned. In both species, top values of energy metabolism and the largest fluctuations were met within the first six-eight weeks. Besides, both RMR and the body weight tended to stabilize and to equal the values found in adult animals.

As to the *Mesocricetus auratus* little ones, the evolution of the energy metabolism was followed up only in the first month of life (Fig. 2). In the first week, a slow raise of this value was observed, while afterwards RMR suddenly climbed up to its maximal value at two weeks' age (25.1 kcal/kg/h). In the little ones of this species, RMR generally reached high values and showed large fluctuations in the first month of life. Great resemblances were found out when comparing the evolution of RMR in both species of genus Mesocricetus. Thus, RMR recorded high values in the first four weeks and also exhibited great fluctuations.

The RMR values recorded in the adults of species *Citellus citellus* and *Mesocricetus newtoni* are given in table 1.

DISCUSSIONS

As already stated in the chapter Results, the newborn individuals of species *C. citellus*, *M. newtoni* and *M. auratus* show resemblances as far as the rest-metabolism rate is concerned. Thus, higher RMR values and larger fluctuations as against the adults are generally recorded in the first weeks of life. Other researches [1] [4] have found out differences of the metabolism intensity related to age. In rats, these authors have recorded high metabolic values and large fluctuations up to the age of 40 days, while in hamsters other authors [3] have found this time interval as being equal to 20 days. In *Mus musculus* L. (the white laboratory mouse), the same metabolic changes have been observed in the first month of life [2].

Although poor researches have been carried out on the energy metabolism in rodent ontogenesis, it may be stated, however, that this parameter has a high value in the first weeks of life. The explanation of this phenomenon is related to the adaptation effort of the young organisms to the environmental conditions which prove to be absolutely new as compared to the maternal body; the lack of the hairy cover, the large volume of the internal organs, the absence of a well-balanced chemical thermoregulation are also valuable explanations in this respect [5].

The large fluctuations described in *Citellus citellus*, even in the period of adult life, demonstrate the low adaptation capacity of this species to the laboratory conditions.

REFERENCES

1. Kleiber M., Smith A. H., Chernikoff H. N., 1956, Amer. Physiol., **186**, 9–12.
2. Myrcha A., 1975, Acta theriol., **20**, *16*, 175–226.
3. Rozaja D. A., Maslennikova L. S., 1968, Tr. In-ta Fizjol. im. Pavlova, **12**, 277–282.
4. Taylor P. M., 1960, J. Physiol., (Lond.), **154**, 153–168.
5. Visinescu Niculina, Nersesian-Vasiliu Cornelia, Radu Domnica, 1965, St. şi cerc. biol., Seria zoologie, **17**, *4*, 347–354.

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IN VITRO MIGRATION INHIBITION OF RAT TUMOR CELLS BY CULTURE SUPERNATANTS OF ANTIGEN STIMULATED LYMPHOCYTES

BY

GEORGE ŞANDRU and LIANA ŞANDRU

In vitro migration of cells from blood clot fragments or from spleen explants of antitumor sensitized rats and of tumor bearing ones was inhibited significantly by syngenic tumor extracts. MIF containing culture supernatants of antitumor sensitized lymphocytes and target tumor cells inhibited the *in vitro* migration both of spleen cells from explants and of syngenic or allogenic tumor cells from capillary tubes suggesting the expression of lymphokine receptors on tumor cell membrane. The migration inhibitory activity was heat stable at 56°C, nondialyzable and susceptible for blocking by L-Fucose.

It was experimentally established that sensitized lymphocytes stimulated *in vitro* by the specific antigen elaborate several soluble factors called lymphokines [3] which play a role in the various manifestations of cellular immunity.

It is not yet known if these different biological manifestations of the lymphocyte products are due to a corresponding number of individual molecules or whether a given factor could have more than one biologic activity depending on the system where it was detected [11].

Migration inhibitory factor (MIF) is perhaps the most convenient lymphokine to assay and its production by antigen or mitogen stimulated lymphocytes is well established. MIF could be produced both by T and B lymphocytes when stimulated under appropriate conditions [12]. Besides macrophages, other cell types have been reported to exhibit MIF receptors such as peripheral leucocytes, erythrocytes, fibroblasts and even tumor cells. Thus mastocytoma cell migration *in vitro* from capillary tubes was inhibited by MIF containing culture supernatants [2].

In our paper we report the *in vitro* induced inhibition of tumor cell migration in rats by using the culture supernatants of antigen stimulated antitumor sensitized syngenic lymphocytes.

MATERIAL AND METHODS

(a) Animals: Inbred R rats immunized against the syngenic B1R tumor by a subcutaneous inoculation of 5×10^5 tumor cells in complete Freund's adjuvant; after 3 weeks from immunization, rats were sacrificed by ether and blood, lymph nodes and spleen were harvested.

(b) Lymph node cell preparation: Cells were obtained by teasing lymph nodes in TC-199 medium (Difco, Michigan, USA) containing 0.02 M HEPES at pH 7.2–7.4, 100 U/ml penicilin, 100 μg/ml streptomycin and 10% fetal calf serum (FCS) (Difco). Cell suspension was centrifuged once at 250 g and sediment suspended in enriched medium at 10^7 cells/ml.

(c) Tumors: B1R ascites tumor appeared as a spontaneous mammary tumor in R rats being then converted in ascitic form and maintained syngenically. The ascites fluid harvested in the 10th day of tumor grafting was washed 2 times in PBS at 250 g centrifugation and the sediment was suspended in culture medium at 10^5/ml concentration.

(d) Preparation of lymphocyte-tumor cell mixed culture. 1 ml of lymph node cell suspension and 1 ml of tumor cell suspension were seeded together in pirex glass tubes of 16 × 150 mm which were incubated at 37°C for 24 h in a water satured atmosphere containing 5% CO_2. Then the culture fluids were centrifuged at 250 g and the supernatants were tested for MIF activity after enriching with 5% FCS; sometimes the culture supernatants were dyalized against 100 v TC medium for 24 h or were heated at 56°C for 30 minutes before testing.

(e) Capillary tube method for ascites tumor cell migration *in vitro*: This was performed as described previously [5]. Shortly the B1R ascites tumor cells washed once in TC medium by centrifuging at 75–100 g was suspended in FCS enriched medium (10^8/ml). Hematocrit capillary tubes of 1.2 mm O were filled by cell suspension, seeled by flame, centrifuged for 1 minute at 75–100 g and cut at cell-fluid interface. The capillary tube fragments containing tumor cells were mounted by silicon grease in the migration chambers and control and assay medium was added. After 24 h of incubation at 37°C the migration areas were projected on Whatman paper for weighing purpose. Arithmetic mean, standard deviation (SD) and migration index (MI) were performed. MI was calculated as percentage of control, MI = (X Area/Control Area) × 100.

(f) The blood clot fragment method: This was performed as described elsewhere [6], [8].

(g) The spleen explant method: A modified Svejcar and Johanovsky technique [10] was performed as published previously [7].

(h) Migration chambers: These were constructed from glass rings of 1.5 cm O and 0.15 cm height mounted upon glass plates by warm paraffin. The upper edge of the migration chamber wall was greased by silicon grease; after filling up by culture medium the chambers were covered by coverslips.

RESULTS

The *in vitro* migration of cells from blood clot fragments or from spleen explants of antitumor sensitized rats was significantly inhibited in presence of the syngenic tumor extracts. When similarly tested the B1R ascites tumor bearing R rats exhibited a significant antitumor directed immunity excepting the terminal stages of the tumor. Moreover the *in vitro* migration capacity of cells from tumor bearing animals decreased with tumor age increase even in absence of antigen. (Fig.1).

Concordant results have been found by using the indirect assay with both methods mentioned above. Besides it has been found that the supernatants of antitumor sensitized lymphocytes cultured in presence of target tumor cells inhibited significantly the tumor cell migration from capillary tubes both of the syngenic B1R and of the allogenic Guérin ascites tumors. The inhibitory activity was resistant to heating at 56°C, to dyalization and could be blocked by adding L-Fucose to cultures (Table 1, Fig. 2).

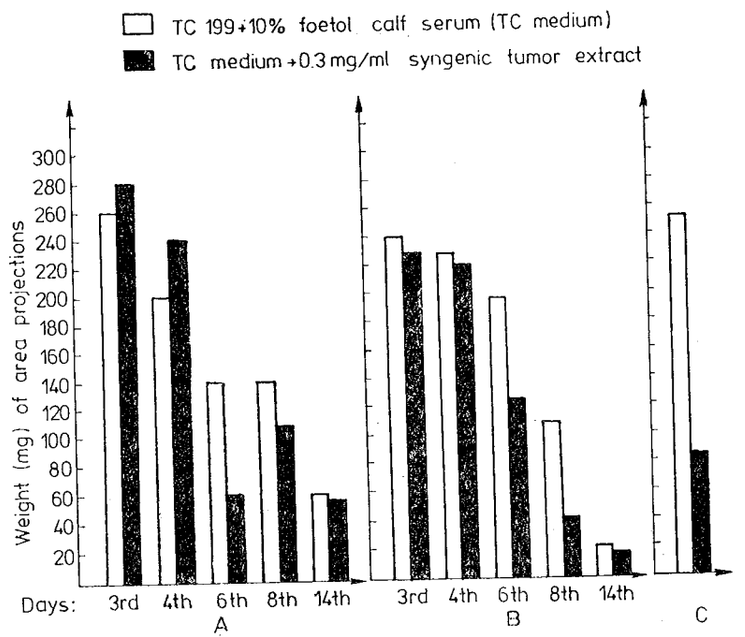

Fig. 1. — *In vitro* detection of tumor directed immunity in tumor bearing rats and experimentally sensitized ones by using the direct MIF assay method.

A: Cell migration from blood clot fragments of B1R ascites tumor bearing R rats;
B: Spleen cell migration from explants of B1R ascites tumor bearing R rats;
C: Spleen cell migration from explants of anti B1R sensitized R rats

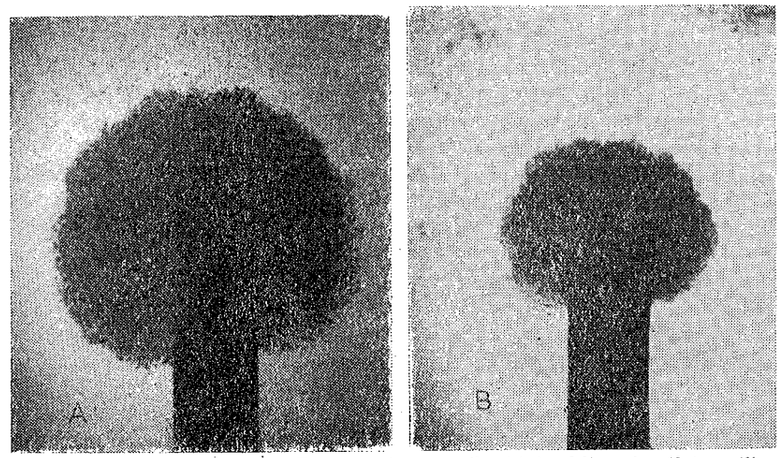

Fig. 2. — Migration areas of B1R ascites tumor cells obtained by using the capillary tube method.

a, in control supernantant fluid;
b, in MIF containing supernatant fluid. Magnification 10×.

Table 1

In vitro migration inhibition of tumor cells and of spleen cells by culture supernatants of antigen stimulated sensitized lymphocytes.

Culture Supernatant of:	Migration[g] ± SD	MI
In vitro migration of B1R ascites tumor cells[a]		
NLy + B1R [b]	215.75 ± 6.61	100.00
SLy + BIR [c]	122.77 ± 2.64	56.90
SLy + BIR [d] (inactivated)	120.75 ± 5.24	55.97
SLy + BIR [e] (dyalized)	110.00 ± 2.34	50.98
SLy + BIR [f] (L-Fucose)	198.10 ± 4.83	91.82
In vitro migration of Guérin ascites tumor cells [h]		
NLy + BIR	99.82 ± 3.45	100.00
SLy + BIR	57.05 ± 4.30	57.15
In vitro migration of spleen cells from explants [i]		
NLy + BIR	419.22 ± 3.55	100.00
SLy + BIR	291.97 ± 2.32	69.64

a) the capillary tube method was applied for studying the *in vitro* migration of rat BIR ascites tumor cells; b) Culture supernatant of 5×10^6 normal rat lymphocytes $+ 5 \times 10^4$ BIR ascites tumor cells of 24 h; c) supernatant of 5×10^6 sensitized lymphocytes from anti BIR immune rats and 5×10^4 BIR tumor cells mixte culture of 24 h; d) culture supernatant previously inactivated at 56°C for 30 minutes; e) culture supernatant dyalized against 100 vTC medium; f) L-Fucose 0.01 M has been added to the culture supernatant before testing; g) mean value of weighings (mg) of 4 migration area projections on Whatman paper ± 1 SD was performed; h) *in vitro* migration of Guérin ascites tumor cells was studied by using the capillary tube method; i) the spleen explant method was performed by using normal rat donors.

DISCUSSION

The tumor directed immunity of sensitized rats and of tumor bearing ones has been detected *in vitro* by using the blood clot fragment and the spleen explant methods. The MIF containing supernatants of antigen stimulated sensitized lymphocytes cultures have been tested upon tumor cell migration *in vitro* by using a modified capillary tube technique. The *in vitro* migration of cells from blood clod fragments and from spleen explants of sensitized rats or from tumor bearing ones was significantly inhibited in presence of antigen, the tumor extracts, added to culture medium, as an *in vitro* correlate of delayed hypersensitivity [1]. It is of importance to mention the decreasing migration capacity of cells from tumor bearing rats in absence of antigen which was proportional with tumor age increase suggesting the release in the body fluids of an inhibitory factor for cell migration as it has already been shown [9]. By the indirect MIF assay using the supernatants of antigen stimulated antitumor sensitized lymphocytes it was found apart of spleen cell migration inhibition a similar phenomenon for tumor cell migration *in vitro*. Both the syngenic B1R ascites tumor cells and the allogenic Guérin tumor cells migrating from capillary tubes *in vitro* exhibited a susceptibility

for the inhibitory action of culture supernatants of antigen stimulated lymphocytes. This inhibitory activity was resistant to heating to 56°C and dyalization and was abolished by adding L-Fucose, a sugar known to prevent the interaction of MIF with specific receptors [4]. Recently Cohen et al., [2] reported the *in vitro* induced mastocytoma cell migration inhibition by the MIF containing culture supernatants. These results extend the range of target cells known to respond to lymphokines but raise a question for further investigations regarding the role of lymphokine-tumor cell interaction in the tumor-host relationship.

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REFERENCES

1. Bloom B. R., Bennett B., 1966, Science, **153**, 80.
2. Cohen M. C., Zeschke R., Bigazzi P. E., Yoshida T., Cohen S., 1975, J. Immunol., **114**, 1641.
3. Dumonde D. C., 1970, Proc. Roy. Soc. Med., **63**, 899.
4. Remold G. H., 1973, J. Exp. Med., **138**, 1065.
5. Şandru G., Pecec M., Florescu M., 1971, III Kongres Kancerologa Jugoslavije, 1923.
6. Şandru G., 1975, Eur. J. Immunol., **5**, 729.
7. Şandru G., 1975, Arch. Roum. Path. Exp. Microbiol., **34**, 239.
8. Şandru G., Şandru L., Repciuc E., 1977, Rev. Roum. Biol.-Biol. Anim., **22**. 141.
9. Şandru L., Şandru G., 1977, Rev. Roum. Biol.-Biol. Anim., **22**, 67.
10. Svejcar, J., Johanovsky J., 1961, Z. Immunitaetsforsch., **122**, 398.
11. Tubergen D. G., Feldman J. D., Polloc E. M., Learner R. A., 1972, J. Immunol., **135**, 255.
12. Yoshida T., Sonazaki H., Cohen S., 1973, J. Exp. Med. **138**, 784.

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GENETIC VARIANTS OF HEMOGLOBINS AND TRANSFERRINS ELECTROPHORETICALLY DETERMINED IN CARPATHIAN GOAT *(CAPRA HIRCUS* L.*)*

BY

EUGENIA MILOVAN and I. GRANCIU

Hemoglobins and transferrins in Carpathian goats of Neamț region of Romania were studied. Blood samples were collected from 104 goats. By analysis of electrophoregrams in starch gel two allelic genes were identified at hemoglobin locus. The gene frequency was 0.928 for gene Hb A and 0.072 for gene Hb B.

The most frequent was the homozygous genotype Hb A (0.8611). No individuals possessing Hb B/B were identified.

At transferrin locus there were also two allelic genes present : Tf A (0.6971) and Tf B (0.3029). All genotypes formed by them were identified : Tf A/A (0.4859), Tf B/B (0.0917) and Tf A/B (0.4223). The tendency to fixation of gene Hb A in this breed is noticed.

Electrophoretic studies on different blood components and body fluids in animals have revealed the existence of a variation of these proteins. Family analysis demonstrated the genetic origin of this variation.

There are few studies in goats in this field. In 1958 Ashton and McDougall [2] noticed the presence of genetic polymorphism in serum transferrins of goats.

Later, in 1964 Bernhardt [3] identified the hemoglobin heterogeneity in goats. Analogous studies have been published by Watanabe in 1965 [5].

In this paper the results of a study on a group of Carpathian goats of Romania are presented. The idea was to initiate an inventory of these biochemical characters and to determine the genes and genotypes frequency.

MATERIAL AND METHODS

A total of 104 Carphatian goats of Neamț region of Romania's Eastern Carphatian mountains were studied.

The blood samples were taken on anticoagulant solution and centrifugated in laboratory for plasma and red cell separation.

For the study of hemoglobins the red cells were three times washed in saline solution and centrifugated. Prior to electrophoresis three times washed red cells were treated with distilled water 1/20 to obtain a proper hemolysis. The detection of hemoglobin types was done by electrophoresis in starch gel in a discontinuous system of buffers identical to that described by G. C. Asthon [1].

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The transferrin types were also determined by starch gel electrophoresis by the method developed by F. K. Kristjansson [4] for prealbumins in pigs and modified by Jamieson for transferrin studies in cattle. Potatoes starch partially hydrolysed in this laboratory was used in 10.5% of gel composition : the buffer consisted of Tris (hydroxymethyl aminomethan) and citric acid at 7.9 pH.

Samples were inserted at a distance of 9 cm of the cathodic end of the gel. An electric power of 18–20 V per 1 cm of gel length was used.

The electrophoresis took four hours for hemoglobins and six hours for transferrins.

RESULTS AND DISCUSSIONS

At hemoglobins locus two alleles, Hb A and Hb B, were identified. The identified genotypes were also two of three possible because the homozygous Hb B/B was not present in the animals studied.

In table 1 alleles, gene frequency and genotypes are presented. The Hb A type has the highest gene frequency over 92%. The type Hb B has a very low presence in Carpathian goats (7%).

Table 1

Gene frequency and genotype distribution of hemoglobin types in Carpathian goat

Hb allele	Gene frequency and standard deviation	Identified genotypes	Estimated frequency	Genotype distribution	
				observed	estimated
A	0.928±0.0180	AA	0.8611	89	89.544
		AB	0.1338	15	13.936
B	0.072±0.0170	BB	0.0051	—	0.580
		Total	n=	104	104.060

Genotypes directly read from electrophoregrams have also a high frequency for the homozygous type Hb A/A (86%).

The heterozygous Hb A/B type had a low frequency (13%) and type Hb B/B was absent.

One can estimate that in goats of the Carpathian type from Romania the process of substitution of allele Hb B is advanced and probably has the tendency to fixate the type Hb A at Hb locus.

At present one can not evaluate the real consequences of this process on goat breeding in this region.

At transferrin locus two alleles were identified : Tf A and Tf B. Electrophoregrams of 104 serum samples revealed the presence of three phenotypes of Tf according to table 2.

Table 2

Gene frequency and genotype distribution of transferrin types in Carpathian goat

Tf allele	Gene frequency and standard deviation	Identified genotypes	Estimated frequency	Genotype distribution	
				observed	estimated
A	0.6971±0.0318	AA	0.4859	53	50.5336
		AB	0.4223	39	43.9192
B	0.3029±0.0319	BB	0.0917	12	9.5368
			Total	n=104	103.9896

The gene Tf A had a frequency two times as great (69%) as of allelic gene Tf B (30%). All possible genotypes were identified. Their rate is variable. Homozygous genotypes Tf A/A had a frequency higher than heterozygous Tf A/B. Very low was the frequency of Tf B/B (9%).

The Carpathian goat showed a variation at Hb and Tf loci genetic controlled and expressed by two allelic codominant genes. In the present stage of improvement genotype and gene frequencies are in favour of the types A at both loci with a variation of homozygous and heterozygous types.

REFERENCES

1. Ashton G. C., 1963, Nature, **198**, 1117–1118.
2. Ashton G. C. and McDougall E. J., 1958, Nature, **182**, 945–946.
3. Bernhardt D., 1964, Dt. Tierdrzt. Wschr. **71**, 461–462.
4. Kristjansson F. K., **131**, Science, 1681.
5. Watanabe S. et al., 1965, Proc. Japan. Acad., **41**, 326.

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Profesor Dr. THEODOR BUȘNIȚĂ

24. August 1900 — 18. August 1977

Professor Dr. Theodor Bușniță wurde am 24. August 1900 in Chișinău — UdSSR geboren. Nach Abschluß der Reifeprüfung im Jahre 1919 absolvierte er zwischen den Jahren 1920—1928 die naturwissenschaftliche Fakultät und die landwirtschaftliche Akademie in Cluj. Den Doktortitel erhielt Professor Bușniță in Biologie im Jahre 1932 in Bukarest, wobei seine Hauptspezialität vergleichende Anatomie der Fische, seine Nebenspezialität theoretische und angewandte Ichthyologie waren.

Nachdem Professor Bușniță in den Jahren 1921—1923 Präparator und in den Jahren 1924—1928 Assistent und Arbeitschef am Zoologischen Institut der Universität Cluj war, übersiedelte er an die Universität Bukarest, wo er bis zum Jahre 1938 am Laboratorium für Tiermorphologie tätig war.

Im Jahre 1945 wurde er zum Dozenten an der Tierärztlichen Hochschule ernannt, wo er seine Tätigkeit bis zum Jahre 1948 ausübte; im gleichen Jahr wurde er zum Professor und Dekan der Fakultät für Fischkultur in Constanța berufen.

Im Jahre 1951 wurde er zum Direktor des Institutes für Fischereiforschung in Bukarest ernannt wo er bis zum Jahre 1953 blieb in welchem er zum stellvertretenden Generaldirektor der Generaldirektion für Fischindustrie des Lebensmittel-Ministeriums berufen wurde. Im Jahre 1956 wurde er in das Institut für Biologie der Rumänischen Akademie berufen, wo er zuerst als Sektionschef, dann als wissenschaftlicher Direktor bis zum Jahre 1965 wirkte, im gleichen Jahre tritt Professor Bușniță in den Ruhestand.

Neben seiner Universitätslaufbahn und seinen rein wissenschaftlichen Arbeiten zeigte sich sein organisatorisches Talent noch im Jahre 1928 durch die Gründung und Organisierung der ersten Fischerei-und Fischkultur-Schule des Landes in Giurgiu, deren Direktor er in den Jahren 1928—1930 war und durch die Gründung der Fakultät für Fischkultur in Constanța im Jahre 1948, die heute in die Universität Galați eingereiht ist.

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Professor Bușniță fühlte sich sein ganzes Leben mit der angewandten Wissenschaft, insbesonders mit der Ichthyologie und Fischkultur verbunden. Aus diesem Grunde übernahm er im Jahre 1930 eine Stelle als Biologe bei der staatlichen Fischereiverwaltung, wo er als Schüler und Mitarbeiter des berühmten Biologen und Ichthyologen Dr. Grigore Antipa in Kürze zum delegierten Direktor und dann zum Direktor der staatlichen Fischereiverwaltung ernannt wurde.

Diese vielseitige Tätigkeit als Wissenschaftler, Pädagoge und Organisator verhinderten Professor Bușniță aber nicht in den Jahren 1925—1956 45 Arbeiten von großem wissenschaftlichen universellen Werte, über die Anatomie und Hystologie der Fische, die Ichthyologie und allgemeine Fischkultur zu veröffentlichen. Vom Augenblicke seines Überganges zum Institut für Biologie „Traian Săvulescu" der Akademie im Jahre 1956, widmete er sich ganz der Wissenschaft und publizierte noch bis kurz vor seinem Tode allein oder in Zusammenarbeit mit seinen Schülern über 65 Arbeiten. Seine letzte wissenschaftliche Arbeit „20 Jahre wissenschaftliche Forschung in Ichthyologie und Fischkultur auf der ganzen Donau und in Rumänien", die bei der Festsitzung des 20-jährigen Bestandes der Internationalen Forschungsgemeinschaft zum limnologischen Studium der Donau im Herbste des Jahres 1976 in Sofia vorgelegt wurde, synthetisierte in komplexer Weise alle wissenschaftlichen Forschungen auf der Donau in den letzten 20 Jahren und krönte so eine fruchtbare wissenschaftliche Tätigkeit auf diesem Gebiete. Ihre höchste Anerkennung fand dieselbe aber in der Veröffentlichung seiner Arbeit von großem praktischen Wert „Die Fischkultur in Brackgewässern" in Rom durch die FAO. Dieselbe wurde an alle Fischerei- und Fischkulturzentren der Welt verteilt.

Als Krönung seiner fruchtbaren wissenschaftlichen und angewandten Tätgkeit wurde Professor Bușniță im Jahre 1955 zum korrespondierenden Mitglied der Akademie ernannt. Im selben Jahre organisierte er, die Hydrologische Kommission deren Vizepräsident er bis zum Jahre 1963 war. Nach dem Tode des Präsidenten dieser Kommission, Akad. Tr. Săvulescu war er bis zum Jahre 1965 Präsident dieser Kommission.

Vom Jahre 1957 bis 1967 war Professor Bușniță der Vertreter Rumäniens in der damals gegründeten Internationalen Vereinigung zum limnologischen Studium der Donau, als hauptsächlichster Begründer von rumänischer Seite her und seit 1959 war er auch Mitglied der Internationalen Limnologischen Gesellschaft.

Im Jahre 1963 wurde Professor Bușniță mit dem Arbeitsorden zweiter Klasse für außerordentliche wissenschaftliche Verdienste ausgezeichnet.

Die rumänische hydrobiologische und fischereiwirtschaftliche Wissenschaft hat mit dem Tode Professors Theodor Bușniță einen schwer zu ersetzenden Verlust erlitten.

Er hinterläßt eine große Anzahl von Schülern, Doktoren und Doktoranden, sowie Fischereitechniker und Praktikanten, die seine Gedanken zum Wohle der rumänischen Wissenschaft auf diesem Gebiet weiterentwickeln werden.

Ludwig Rudescu

AVIS AUX AUTEURS

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

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

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