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# L'ŒUVRE UNIVERSITAIRE ET CYTOLOGIQUE DE DIMITRIE N. VOÏNOV (1867—1951)

PAR

RADU CODREANU

The paper summarizes the universitary and social work of Dimitrie N. Voïnov (1867—1951), the founder of the chair of Animal morphology (1892—1937) at the Bucharest University, after studies with the French zoologists H. de Lacaze-Duthiers and Yves Delage. His research work started in histophysiology on the excretory and phagocytic properties of the ectoparasitic oligochaete *Branchiobdella* and odonate larvae by means of physiological injections with vital stains (1896, 1898). Experimentally he also proved the antitoxic function of the interstitial gland in homeotherms (1905). As a pioneer in the cytological study of the insect spermatogenesis (1902), Voïnov came to the valuable discovery in the European *Gryllotalpa* of the first *aneuploidy* known in animals (1914) and on the part of mitochondria in inheritance (1916). His chief cytological results concern the general presence of dictyosomes as Golgi apparatus in all animal cells (1934). He pointed out the dictyosomes secretory functions, calling them *ergastoblasts*. These facts became fully confirmed by their further ultrastructural study in electron microscopy.

La commémoration en cette année de trois décennies depuis la mort de Dimitrie Voïnov, survenue par un regrettable accident le 7 juillet 1951, nous offre l'occasion de rendre hommage à son rôle de promoteur du développement moderne de la biologie roumaine et de relever les priorités souvent méconnues de ses recherches.

Sa vie se déroula dans un perpétuel effort de dépassement scientifique, qu'il relia dès sa jeunesse aux aspirations sociales les plus avancées. Né le 6 février 1867 à Jassy, il eut l'avantage d'un milieu familial élevé, ayant été adopté par un proche-parent, le juriste Nicolae Voïnov personnalité politique passionnément attachée aux idées révolutionnaires de 1848 qui avaient engendré l'union des Principautés Roumaines. Au lycée « Institutele-Unite », où enseignaient surtout des professeurs de l'Université, D. Voïnov fut collègue de classe avec Grigore Antipa et Emile Racovitza, dont l'illustre carrière de naturalistes les réunira tous les trois à l'âge mûr comme membres de l'Académie Roumaine. Encore collégiens, leur esprit va s'enflammer pour deux courants d'idées qui éclataient à l'époque : l'évolutionnisme par suite des cours éloquents du savant géologue Grigore Cobălcescu et le socialisme puisé dans leurs lectures du périodique « Contemporanul ».

Après le baccalauréat et son service militaire, D. Voïnov rejoint son ami E. Racovitza à Paris, où ils se partagent entre les études de sciences naturelles et une vive activité dans le mouvement socialiste français avec tout un groupe d'autres étudiants roumains figurant sur des tableaux photographiques conservés jusque de nos jours. Il subsiste de même la carte de Voïnov comme délégué des ouvriers typographes roumains au congrès de la deuxième Internationale (1889). A la Sorbonne, il fréquente les mêmes maîtres que Racovitza, notamment Henri de Lacaze-Duthiers

avec ses collaborateurs, Yves Delage et Georges Pruvot, qu'il suit également dans les laboratoires maritimes de Roscoff sur la Manche et de Banyuls-sur-Mer à la Méditerranée, où étudiait pareillement Ion Cantacuzino. Promu licencié ès Sciences Naturelles en 1890, Voïnov fait des séjours de travail dans d'autres stations méditerranéennes, telles Villefranche et Naples en quête d'un sujet de thèse de doctorat, tâtonnant une étude des ascidies, ainsi qu'il en ressort de ses lettres à Racovitza. Il y revoit deux autres compatriotes, Paul Bujor qui préparait sa thèse chez Carl Vogt à Genève et Gr. Antipa, devenu le disciple d'Ernest Haeckel à l'Université d'Iéna.

Cependant Voïnov doit regagner son pays, car une chaire de Zoologie et morphologie venait d'être séparée de la chaire de Physiologie d'Alexandru Vitzou à l'Université de Bucarest, et sa famille l'appelle à participer au concours institué dans ce but à Jassy, devant un jury dominé par le prof. Léon C. Cosmovici. Malgré la compétition de Nicolae Léon et Paul Bujor, c'est D. Voïnov qui fut déclaré gagnant (1892), devenant à vingt-cinq ans titulaire d'un enseignement biologique fondamental, qu'il allait honorer pendant près d'un demi-siècle jusqu'à sa mise à la retraite (1937).

Parallèlement à ses lourdes tâches d'organisation à travers les vicissitudes de dizaines d'années passées dans des locaux impropres, Voïnov se voue avec ténacité à une mission complexe. Demeuré fidèle à notre mouvement ouvrier, il va propager des conceptions matérialistes par ses articles publiés dans le recueil « Literatură şi Ştiinţă » (1893, 1894) de son ami, C. Dobrogeanu-Gherea et dans « Convorbiri literare » (1906, 1907) à la défense du transformisme, injustement dénié par le médecin physiologiste N. Paulesco. Avec I. Cantacuzino, il va se lancer dans de véhémentes protestations contre les répressions sanglantes de 1907. D'autre part, pour assurer les progrès de la Faculté des Sciences, il appuie la nomination de I. Athanasiu à la chaire de Physiologie animale (1905) et d'Em. Téodoresco à celle de Physiologie végétale (1907). Longtemps il épaula les actions que le réputé chimiste dr. C. Istrati déployait au profit de la Société des Sciences de Bucarest et entreprit avec ses collègues I. Athanasiu et I. Cantacuzino la fondation de la filiale roumaine (1906) de la Société de Biologie de Paris. Grâce à cette adhésion, de nombreux résultats des recherches biologiques roumaines trouvèrent leur prompte impression jusqu'à la dernière guerre mondiale, dans les Comptes Rendus universellement répandus de la prestigieuse Société française.

Mais sa situation aisée et ces absorbantes activités culturelles et sociales ne le détournent nullement de la recherche scientifique. Là Voïnov a le mérite de poursuivre une orientation de plus en plus moderne, étant plutôt influencé par l'exemple d'Yves Delage, auquel il gardait une admiration ineffaçable, et s'éloignant manifestement du classicisme de Lacaze-Duthiers. Les contacts avec les célèbres embryologistes russes, A. Kowalevsky et E. Metschnikoff au cours de séjours communs dans les laboratoires maritimes français, incitent Voïnov à appliquer la méthode des colorations vitales à l'étude de l'excrétion chez les invertébrés. Ces recherches, qu'il effectua en Roumanie, lui permirent la mise en évidence d'un filtre cilio-phagocytaire chez *Branchiobdella*, oligochète ectoparasite de l'écrevisse (1896, [15]) et des corrélations physiologiques entre l'appareil digestif, les cellules péricardiales et les tubes excréteurs des insectes odonates (1898, [16]). Ses résultats, consignés dans le grand traité de physiologie comparée de H. Winterstein (1911, [27]), sont contemporains de travaux du même ordre des savants notoires, tels L. Cuénot chez les insectes orthoptères (1896) ou P. Marchal chez les crustacés décapodes (1892).



Convaincu de la portée du perfectionnement technique dans l'investigation des processus cellulaires, Voïnov entreprit un voyage d'études dans plusieurs centres universitaires d'Allemagne: Heidelberg, Freiburg... (1895—1896). Il fait paraître ensuite son livre « Principii de microscopie » (1900, [17]), conçu d'une manière particulièrement méthodique afin d'implanter les pratiques histologiques dans les laboratoires roumains. L'originalité de cet ouvrage consiste dans la large part qu'il assigne à l'histophysiologie au moyen des procédés d'injections physiologiques de colorants destinés à l'analyse comparée des fonctions digestives, excrétrices et phagocytaires le long de l'échelle animale. Signalons encore que peu après la découverte par P. A. Bouin et P. Ancel de l'activité hormonale de la glande interstitielle, Voïnov démontre expérimentalement son rôle antitoxique (1905, [19]), qu'il serait intéressant de mettre en rapport avec le facteur de diffusion hyaluronidasique, isolé ultérieurement du testicule par Duran Reynals (1928).

Entre temps, l'essor de la génétique par le mendélisme suscite un courant mondial pour l'étude cytologique des phénomènes de reproduction et Voïnov se trouve parmi les pionniers des recherches sur la gamétogenèse. Après des travaux sur la formation des spermatozoïdes atypiques chez les coléoptères et les papillons (1903, [18]), il approfondit la spermatogenèse de la courtillière *(Gryllotalpa gryllotalpa)*, aboutissant à un faisceau de résultats hautement significatifs (1914, 1925, [20], [23]). En effet, tout en conservant la même morphologie, les *Gryllotalpa* d'Europe possèdent un nombre différent de chromosomes suivant la localisation géographique des populations : 2n = 12 en Allemagne, 2n = 14 en Roumanie et 2n = 15 dans l'Italie méridionale. Cette variation géographique du caryotype, pleinement confirmée depuis (Grassé, 1966, 1973, [4], [5]) représente la première *aneuploïdie* connue dans une espèce animale, ayant précédé d'une quinzaine d'années la fameuse découverte d'Artom (1931, [1]) des cas de polyploïdie complète (euploïdie) chez le crustacé *Artemia salina*. En outre, ayant identifié dans les spermatocytes primaires de *Gryllotalpa* une paire de chromosomes homologues hétéromorphes, Voïnov constate leur *polarité variable* lors de la méiose et l'interprète comme une illustration cytologique de la disjonction mendélienne (1914, [21]).

Ces travaux de cytogénétique l'amènent à s'occuper des constituants cytoplasmiques, encore peu étudiés à cause de leur fragilité vis-à-vis des techniques courantes. Il fait d'abord connaître, toujours à la méiose des spermatocytes de *Gryllotalpa*, un mécanisme très précis de division des mitochondries, qu'il appelle *chondriodièrese* (1916, [22]) selon le terme des cytologistes italiens Giglio-Tos et Granata (1908). Cette disposition équatoriale des « chondriosomes » entourant les chromosomes et leur étirement subséquent porte Voïnov à attribuer un rôle héréditaire aux mitochondries, idée qui est actuellement certifiée par la présence de l'acide désoxyribonucléique (ADN) mitochondrial.

Après la Première Guerre mondiale, D. Voïnov et I. Athanasiu réussirent au prix de longs efforts à installer leur laboratoire dans le bel édifice qui venait d'être achevé Splaïul Independentzei (1926). C'est là que Voïnov poursuit ses recherches capitales qui l'ont conduit à éclaircir la véritable nature de l'appareil réticulaire interne de Golgi, en y entraînant toute une série d'élèves. Là-dessus, une grande confusion régnait à l'époque, due surtout aux écoles du botaniste A. Guilliermond (Lyon, 1927, [8]) et du zoologiste M. Parat (Paris, 1928, [12]), qui n'admettaient que deux constituants cytoplasmiques, le chondriome et le vacuome, auxquels ils voulaient ramener les structures golgiennes.

Basé sur une technique rigoureuse, Voïnov démontra irrévocablement (1927—1934, [24], [25]) que l'appareil de Golgi est un organite cellulaire indépendant et général, présent aussi bien chez les invertébrés que chez les vertébrés, sous la forme des corpuscules en croissant ou écailleux discontinus. Ayant une double constitution, chromophile et chromophobe, clairement définie par Voïnov (Grassé et Hollande, 1941, [7]), ils furent désignés comme *dictyosomes* par A. Perroncito (1910) et signalés en fait depuis G. Platner (1889). Leur agencement en un réseau périnucléaire continu, décrit initialement par C. Golgi (1898) dans les neurones, s'explique, d'après Voïnov partiellement par une hypertrophie fonctionnelle, partiellement par les techniques défectueuses d'imprégnation argentique. Il fait remarquer d'ailleurs que S. Ramon Y Cajal lui-même (1914) avait figuré les stades dispersés précédant la formation du réseau.

Assignant aux dictyosomes golgiens une forte activité sécrétoire, Voïnov les nomme *ergastoblastes* [25], capables d'élaborer au contact de leur couche chromophobe divers produits cellulaires : mucus, grains de zymogène, vitellus protéique et même l'acrosome des spermatozoïdes à partir de l'idiozome. Il rapporte également aux dictyosomes ergastoblastiques des constituants cellulaires jusqu'alors énigmatiques, tels les parasomes des cellules glandulaires et les corps vitellins de Balbiani des ovocytes d'araignées, rendus volumineux par leur excessif fonctionnement sécrétoire. Ce fut pour Voïnov l'occasion de fonder une école de cytologistes roumains [26], qui prouvèrent l'existence des dictyosomes golgiens dans différents types de cellules, nerveuses, glandulaires et germinales chez des groupes variés : gastropodes (Margareta Athanasiu), crustacés (Gh. Dornesco, V. Gh. Radu), aranéides (Florica Ionescu-Mezincescu), insectes (I. Steopoe, Victoria Voïnov), amphibiens (Gh. Dornesco, T. Busnitza) etc. Parmi les premiers auteurs étrangers qui confirmèrent la conception de Voïnov sur les éléments golgiens, citons O. Duboscq et P. P. Grassé (1933, [2]) dans une étude approfondie de l'appareil parabasal des flagellés et Odette Tuzet à propos de la spermatogenèse des gastropodes prosobranches (1930, [14]).

Mais la confirmation plénière et décisive vint depuis que la cytologie a passé de la microscopie photonique à l'analyse des infrastructures cellulaires en microscopie électronique [6]. Actuellement, l'appareil de Golgi signifie, tel que l'entendait Voïnov, l'ensemble des dictyosomes, dont l'ultrastructure feuilletée comme une pile de saccules aplatis les distingue nettement de tous les autres organites cellulaires, réticulum endoplasmique, ribosomes, lysosomes, mitochondries, etc. et de même que ceux-ci, ils peuvent être séparés par centrifugation différentielle. Néanmoins, des communications persistent entre ces compartiments, assurant le transport de substance, des courants de vésicules et un flux de cytomembranes au cours du fonctionnement et des biosynthèses cellulaires.



L'activité sécrétoire confère une polarité aux dictyosomes golgiens, qui élaborent sur leur face concave des produits dérivés de l'ergastoplasme et rejettent continuellement des vésicules périphériques aboutissant à l'exocytose à travers le plasmalemme. Des auteurs récents, Franke et al. (1971, [3]), Palade (1975, [11]), Morré et Ovtracht (1977, [10]) soulignent le rôle dynamique de l'appareil de Golgi dans la différenciation et l'interconversion des endomembranes constitutives de la cellule. Il apparaît apte d'une large gamme de sécrétions depuis la mucine, des polysaccharides et glycoprotéines jusqu'à des structures complexes comme le perforateur des spermatozoïdes et le filament des nématocystes des coelentérés ou de la spore des microsporidies.

Quant au vacuome, il a perdu la signification exagérée d'autrefois, étant réduit à un réceptacle de réserves cellulaires. C'est ainsi que l'ouvrage synthétisant la conception de Voïnov, « Structures ergastoblastiques » [25] garde une remarquable actualité malgré sa date lointaine (1934). Ses résultats, il les publiait à l'état préliminaire dans les Comptes Rendus de la Société de Biologie à Paris et ultérieurement in extenso dans les Archives de Zoologie expérimentale et générale, dirigées par son vieil ami, Emile Racovitza. Dans la mise au point sur la cellule du Précis de Biologie générale sous la direction de P. P. Grassé, l'auteur A. Hollande (1966, [9]) fait débuter le chapitre sur l'appareil de Golgi en confrontant deux figures : l'une de Voïnov sur les dictyosomes épars, l'autre périmée de Golgi avec le réseau périnucléaire interne. En dépit de cet édifiant témoignage, nombre de traités omettent à l'étranger l'indéniable apport de Voïnov.

Depuis V. Babeş, dont D. Voïnov a pris la succession à l'Académie Roumaine, sur le rapport dressé par E. Racovitza et I. Cantacuzino (1927, [13]), un mouvement s'est graduellement amplifié en faveur de la biologie cellulaire, qui est devenue actuellement un domaine actif de la vie scientifique en Roumanie. Les priorités réelles de l'œuvre cytologique de D. Voïnov s'y inscrivent brillamment et nous avons le devoir de les faire définitivement intégrer dans le patrimoine scientifique international.

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# *CARPOAPSEUDES KUDINOVAE* N. SP. IN THE FRENCH WATERS OF THE ATLANTIC

BY

MIHAI BĂCESCU

Species *Carpoapseudes kudinovae* n.sp. is described after a material collected by Biogas Campaigns in the Gulf of Gascogne and sent to the author for study by the Britain Oceanological Center (CENTOB). A dichotomic key of the eight species known so far as belonging to the genus is given.

*Material* 1 adult ♂ (14.2 mm) and 1 ♂ p (12 mm).

Biogas VI, DS — 71, Gulf of Gascogne (47°43′3″N ; 8°33′8″W), 2194 m. Dredge, 1977 ; 1 ♀ with oostegites (16.2 mm). St. C.P. Biogas.

*Description of male type*. Body (Fig. 1A) relatively short, 5.7 times longer than the maximum width attained at the level of the first free peraeonite. Integument soft, slightly calcified, bright white and almost glabrous all along. Carapace 1.3 times longer than wide, with the usual carving in the posterior half ; it is almost twice wider at the level of the swellings of the respiratory chambers than the area at the back of the ocular lobes. Between these areas a slight smooth inflation without a trace of spine. The ocular lobes well separated, wide at their basis, end by a long spiny apophysis. Rostrum straight, acute-angled triangle shaped, gradually carved medially ; the sides of its basis upright thus marking an excavation between it and the ocular lobe.

The free peraeonites I — IV show medially areolations and transversal depressions (Fig. 1A). On the sides of free peraeonite I one can see the coxal spines of peraeopod II that are about as long as the lateral spines of thoracopods III — V. The other thoracic segments with small antero-lateral tubercles taking even the shape of spines in IV and V and a strong medio-lateral sharp spine ; IV is the longest and VI the shortest. Their sternites show sharp hyposphenia, the largest being placed between chelipeds ; on sternite VI a long genital cylinder (not the cone common to other species) (Fig. I C, D).

Pleonites appear wider than the last peraeonites as a consequence of the long epimeres that suddenly narrow themselves and become sharp spines, curved infero-posteriorly ; at the basis of these spines a bunch of long plumose setae that contrast with the ± glabrous dorsal surface. Pleonites also bear hyposphenia, but short or even with blunt ends.

Telson cylindrical, a bit longer than all the pleonites taken together, rugous, that is with lots of tubercle-shaped eminences or transversal prominences (Fig. 1, $A_1$). Several short rare hairs. Anal opening perfectly circular ; the anal clacks open laterally and show a vertical slit-anus.

Between the peraeopods VII a strong penial organ, long, cylindrical with oval opening (Fig. 1 C and D).

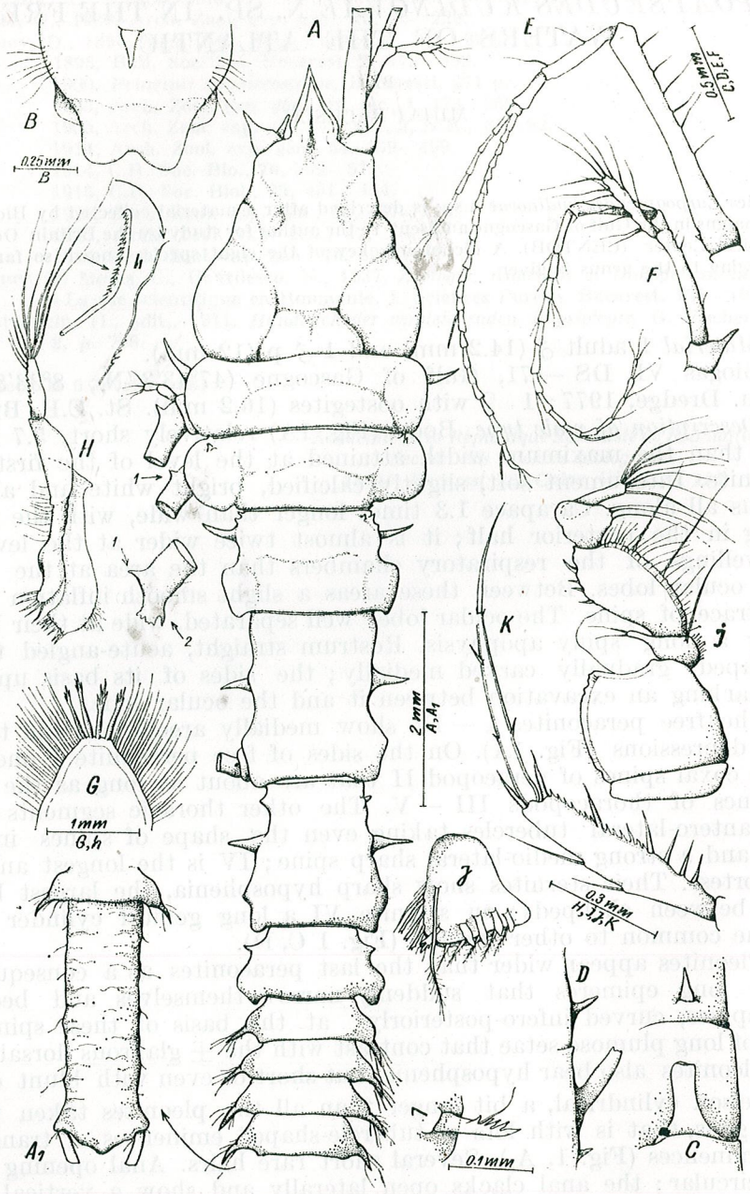


Fig. 1. — *Carpoapseudes kudinovae* n.sp.

A, ♂ seen from above; B, labrum and epistomal spine; C, penial organ and hyposphenia of last but one thoracomer; D, id., lateral view; E, antennula; F, antenna; G, one of the labial lobes; H, maxillula; h, extremity of the long seta of palp; I, maxilliped; J, endite of maxilliped; K, extremity of peraeopod VI; paradactylar claw of peraeopod III.



*Appendages*. Antennula (Fig. 1 E) with a basal article as long as carapace; little flagellum 7-jointed and big flagellum 25-jointed. It shows but 2 aesthetascs in pre-adult ♂ ($A_1$ lacks in adult ♂).

*Antenna* (Fig. 1F) is characterized by a strong sharp apophysis on the inner and terminal side of the first basal article; these symmetrical apophyses are seen normal from above like two little horns between basis $A_1$ and rostrum; its scale shows 13 setae; flagellum 13-jointed.

*Labrum* with soft, curved lip as shown in Fig. 1B, without any prominence in the excavation and with a spine not too long on the epistomal shield.

*Mandible* with 2 strong multicuspidate reddish brown teeth; lacinia with a bunch of dentate laminae on the one side with pars masticatoria weakly developed and a long 3-jointed palp.

Lobe of the *Labium* oval, short, with 3—4 multifid phanerae and a rich set of long fine hairs all around (Fig. 1G).

*Maxillula* (Fig. 1H) of genus type, with 9 setae whose tip is dentate (about more than 20 dents) (Fig. 1h) of three sizes: the 4—5 proximal ones as long as article II of the palp, 5 as long as the palp and the terminal seta as long as the whole maxillula, with twisted end, with finer and fewer dents.

*Maxilla* almost like that of *C. serratispinosus* (Lang, Fig. 28g) with a continuous dentation on the interior side and on the exterior proximal curvature.

*Maxilliped* (Fig. 1I) with 4 retinacles in endite (Fig. 1J).

*Cheliped* much stronger than peraeopod II (Fig. IIA) shows 2 apophyses on the propus and a ± circular excavation on the middle of its inferior margin — auricula; carpus with a dorso-terminal spiniform prolongation and a postero-inferior tubercle; basis with a spiniform apophysis reaching coxa, near which an exopodite with 4—5 setae appears.

Peraeopod II is not adapted for digging (Fig. IIC) and shows plenty of long setae on propus, carpus and merus which is characteristic of the genus; propus just a bit shorter than carpus; the latter is longer than merus, being armed with 3—4 double-serrated spines rather thin than laminous, suddenly appearing as a sharp thread.

Propod 5 times longer than wider (in *C. serratispinosus*, it is only 3 times longer) and shows 3 spines of this type. Basis with a posterior basal tubercle and another one near the insertion of coxa, ventrally. Its exopodite with 5 setae.

*Peraeopod* III with dactyloclaw longer than carpus; on its basis a spiniform tubercle appears (Fig. 1A arrow 1); as the other peraeopods, its basal spine turns round the coxa (arrow 2); all these thoracopods thin and round off the 3 last but one segments and their dactyloclaw becomes considerably longer (Fig. 1K).

The paradactylar spine with 4 denticles (Fig. 1L). The inferior edge of the propus with at least 4 serrate and thin spines.

*Peraeopod* VI (Fig. 1K) with 2/3 of the inferior edge of its propod armed with sharp denticles and 3 serrate and laminate spines; 1/3 proximal with only 2—3 common spines. Pleopods like in Fig. 2E; the distinct seta of endopodite with a largely curved tip (Fig. 2F). Flagelli

of uropods are broken; only the outer one from the second ♂ is present, being 16-jointed.

*Holotype*: ♂ ad. Col. Crustaceans Mus. "Grigore Antipa", no. 518; allotype ♀, ibid., 519.

*Description of female*. 1 specimen with oostegites (16.2 mm). Armature of carapace and of the whole body like in ♂; in addition, in ♀, spiny tubercles on the basis of peraeopods II and III and special phanera on the endopod of pleopods. Integument glabrous, with extremely rare hairs; hyposphenia sharper; soft lip of labrum slightly carved with a smooth contour; 3 aesthetascs on $A_1$.


Fig. 2. — *Carpoapseudes kudinovae* n.sp.
A, cheliped; B, tip of propod of peraeopod II (♀ = 16 mm) with basis of dactyloclaw (d), the flagellate distal spine (e) and the special phanera (f); C, peraeopod II; D, one of the double serrate and acuminate phanera; E, pleopod IV; F, tip of special seta of endopodite, magnified.

Uropods with 23-jointed endopodite and with 17-jointed exopodite. At the tip of propus of peraeopod IV a 4- fid lamina (phanera) appears (Fig. 2B).

*Observations*. The new species is dedicated to the distinguished tanaidologist P.K. Kudinova-Pasternak; it was dredged in the Gulf of Gascogne.



As curious as it might seem, it approaches in the most the morphology of species *C. serratispinosus* from the Gulf of Panama (Pacific Ocean) — in the shape of labrum, of the distinct seta of the endopod of pleopods etc. — and not of the Atlantic species. It differs, however, in the triangular shape of rostrum, the free peraeonite 2 (3 according to Lang) much wider than 3, in another armature of the sides of peraeonite III as well as in lots of microstructural details (paradactylar phanera of peraeopod III, the thin phanera of propod and other length ratios among which the larger merus etc.) and especially in the *auriculation of the palma of propus of chela in the male in reproduction stage* which is missing in Lang's paper, even if he describes a perfectly adult ♂ after the rich armature of aesthetascs of $A_1$.

A remark should be made however, that this auriculation, although also known uniquely in *C. auritocheles* Kud.-Past. and in *C. simplicirostris*, could also be present in the ♂♂ of other species of the genus, but it appears only at the last moulting, that of the reproduction stage. The case of the 2 ♂♂ under study is eloquent enough in this respect; the one of 12 mm lacks the auricular excavation on the propus of chela and has but 3 aesthetascs on the antennula; in the other one of 14.2 mm the excavation is present. The penial cylindrical tube that replaces the cone common to other species as well as the auricular excavation draw the new species near *C. simplicirostris*; while the shape of carapace, of the ocular lobes and especially the exceptional length of the rostral spine and the presence of a single apophysis on the dactylus of the propus and the absence of the coxal spines on peraeopod II and of that from the basis of peraeopods II and III etc. clearly distinguish the new species.

Our species differs from *C. bacescui* Guţu, both by the absence of the auricular excavation in ♂ (checked on ♂ in reproduction stage included in the collection of the "Grigore Antipa" Museum), the practically glabrous integument, another structure of the paradactylar phanera of peraeopod IV and the lack of the spiniform tubercles from the basis of basipodites of peraeopods.

*C. auritochelis* differs from the Atlantic (Romanche trench), in the shape of rostrum and particularly in the lack of the pair of lateral spines in the carapace.

With *C. kudinovae*, the number of species of the genus *Carpoapseudes*, a genus of great depths (2470—7200 m) (exceptionally recorded at 875 m), is raised to 8 and of the Atlantic species to 3.

### KEY FOR THE IDENTIFICATION OF CARPOAPSEUDES SPECIES VALID FOR BOTH SEXES

**1 (2)** Carapace shows 1 lateral spine on the anterior curvature of the respiratory chambers — *C. auritocheles*, Atlantic, Romanche, 7200 m.
**2 (1)** Carapace without lateral spines . . . . . **3**
**3 (6)** Rostrum ± triangular . . . . . **4**
**4 (5)** Ocular lobe extends into one long thin spine, longer than the width of its basis ... *C. oculicornutus* Lang, Kermadek, 2700 m.

**5 (4)** Ocular lobe with spine shorter than its width ... *C. kudinovae* n.sp. Gulf of Gascogne.

**6 (3)** Rostrum like a trapezium or of a similar shape . . . . . **7**

**7 (8)** Rostral median point scarcely sketched (it hardly represents 1/3 of the width of the basis) ... *C. menziesi* Guţu, Peru 4364 m.

**8 (7)** Rostral end at least as long as the width of the basis . . . **9**

**9 (10)** Rostral end like a spine longer than the width of the basis and ocular lobes not sharp ... *C. simplicirostris* Atlantic (NW Ireland) 2200 m.

**10 (9)** Rostral end ± triangular about as long as the width of the basis . . . . . **11**

**11 (12)** Tip of basal (proximal internal seta of the uropodal endopodite ± straight, with a small hirsute proximal area ... *C. longissimus* Pacific Panama, 3570 m.

**12 (11)** Tip of basal (proximal) internal seta sinuous or sickle-shaped, with a double series of dents in the proximal half; the tip draws 2—3 denticles after a long smooth area . . . . . **13**

**13 (14)** Seta clearly sickle-shaped *C. serratispinosus*, Pacific Panama, Alaska 875, 3570 m.

**14 (13)** Seta is only sinuous *C. bacescui* Guţu, Peru, about 4000 m.

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# LE DESSIN DES AILES AU POINT DE VUE TAXONOMIQUE

PAR

EUGEN V. NICULESCU

In this work the author points out that the wing drawing most often supplies a proper taxonomic character and, thus, is able to define the species. There are biologists who wrongly consider "that no specific character is valuable if taken isolately". In fact, a good character of the drawing, as it appears in *N. antiopa*, may, if taken isolately, define the species better than the reproductive isolation, ecology or the chromosomal formula. Further on, the author shows a series of cases in which, on the contrary, the wing drawing is deficient, thus requiring a rigorous determination based upon the genitalia, as, for instance, in Eurytides species of the protesilaus group.

Le dessin des ailes est un élément important pour la taxonomie. Il a attiré l'attention des lépidoptéristes des temps de Linné et a été utilisé, presque en exclusivité, pendant tout le XIX$^{e}$ siècle. C'est le premier élément qui soit pris en considération par le chercheur, qu'il soit débutant ou spécialiste consacré. Les autres caractères sont examinés ultérieurement après que le lépidoptériste ait fait une première « identification » de l'espèce d'après le dessin. Le plus souvent l'identification est correcte en utilisant seulement le dessin, mais il y a un grand nombre d'espèces où le dessin est déficient et l'identification correcte se fait à l'aide des autres éléments (les palpes, les antennes, la touffe frontale de poils et surtout l'armature génitale).

La déficience du graphisme est due à plusieurs causes :

1. Parfois le dessin manque, les ailes étant monochromes.

2. Chez un bon nombre d'espèces le dessin est exprimé très clairement, mais il est identique ou presque, au dessin d'une autre espèce (convergences).

3. Parfois il y a une grande variation individuelle et le caractère spécifique se « perd » dans la multitude de taches qui varient à l'infini.

Par conséquent le dessin a une valeur inégale au point de vue taxonomique.

Pour mieux comprendre le sens du mot inégal comparons le dessin des espèces *Nymphalis antiopa* et *N. polychloros*, ou *Arctia caja* et *A. villica* ou *Papilio torquatus* et *P. anchisiades* d'une part et les 11 espèces d'*Eurytides* (le groupe protesilaus) d'autre part.

Quoique *antiopa* et *polychloros* fassent partie d'un même genre, le dessin et le coloris sont toutefois profondément différents et aucune confusion n'est possible. *Antiopa*, par la couleur noirâtre de ses ailes et la bordure jaune se reconnaît rapidement et se distingue facilement parmi les milliers d'autres papillons. Ce dessin définit excellement l'espèce puisqu'il ne se répète chez aucune autre espèce.

Ainsi l'opinion de certains biologistes qui affirment qu'aucun des caractères d'espèce, pris isolément n'est valable, est évidemment erronée.

Pourquoi le dessin d'*antiopa* n'est-il pas valable? A mon avis, ce caractère a une grande valeur taxonomique, au niveau spécifique, puisqu'il est présent chez tous les individus et ne se rencontre chez aucune autre espèce; il définit seulement l'espèce *N. antiopa* et la définit beaucoup mieux que l'isolement reproductif, l'écologie ou la formule chromosomique. Il suffit d'examiner seulement l'aile antérieure pour nous prononcer immédiatement et avec toute certitude sur l'appartenance du spécimen à l'espèce *N. antiopa*. Dans ce cas le dessin a une grande valeur taxonomique car tout seul il permet l'identification et la délimitation de l'espèce.

Pour comprendre encore mieux ce phénomène nous examinons maintenant le dessin chez *N. xanthomelas* et *N. polychloros*. Cette fois les deux espèces de *Nymphalis* sont beaucoup ressemblantes par le graphisme et l'identification de l'espèce. *N. xanthomelas* est assez difficile. Pour une détermination correcte il est nécessaire d'utiliser aussi d'autres caractères morphologiques (les pattes et l'armature génitale, ainsi que la morphologie et l'écologie des chenilles). Donc le dessin chez xanthomelas est déficient, il n'a plus une grande valeur taxonomique comme chez *antiopa*. Au contraire chez *P. torquatus* et *P. anchisiades*, *Limenitis populi* et *L. reducta*, *Vanessa cardui* et *V. atalanta*, *Arctia caja* et *A. villica*, etc. le dessin a une grande valeur taxonomique.

Nous examinons maintenant le genre *Eurytides* à savoir le groupe protesilaus. Dans ce groupe il y a 11 espèces plus ou moins ressemblantes dans l'habitus. *E. protesilaus*, *E. telesilaus*, *E. helios*, *E. earis*, *E. molops*, *E. haeterius*, etc. s'identifient très difficilement d'après l'habitus et pour une détermination correcte il faut utiliser la touffe frontale de poils et surtout l'armature génitale; c'est le même problème que chez *xanthomelas* où le dessin est déficient. En opposition avec le groupe *protesilaus*, nous avons, dans le même genre les groupes *thyastes* (6 espèces), *dolicaon* (7 espèces), *marcellus* (10 espèces) et *lysithous* (18 espèces) où le dessin est si varié interspécifiquement et bien défini que l'espèce peut être aisément et rapidement identifiée sans recourir à un autre caractère. Les cas d'espèces avec habitus identique ou se ressemblant beaucoup nous les avons encadrés dans le phénomène de *convergence interspécifique* [3] ceux avec habitus nettement différent dans le phénomène de *divergence intragénérique* [3].

Un problème important pour la taxonomie est aussi le problème de la variation individuelle et géographique. Chez certaines espèces (*N. antiopa*, *I. io*, *P. brassicae*, *V. atalanta*, *A. paphia*, *E. telesilaus*, etc.) la variation individuelle est très réduite. Par contre, chez les espèces du genre *Melitaea*, surtout chez *M. didyma*, *M. phoebe*, *M. cinxia*, *M. athalia*, etc. elle est extraordinairement développée ce qui masque, parfois, le caractère spécifique. Par la fusion de certaines taches et stries apparaissent des taches et bandes plus grandes que d'habitude et par le développement de certains pigments apparaissent des couleurs « nouvelles » (mélaniques ou albiniques) qui ne sont pas caractéristiques à l'espèce dont il s'agit et masque ainsi les véritables caractères spécifiques. Et toutefois ces grandes taches noires, rougeâtres ou blanchâtres insolites ne sont pas — chez ces espèces — des caractères spécifiques mais aberrants, tandis que chez *E. telesilaus* les taches jaunâtres des ailes postérieures, dans le voisinage de la queue, sont spécifiques. La variation géographique est,



elle aussi, très souvent assez grande. Il y a des races géographiques où le dessin est beaucoup plus différencié que chez les espèces d'*Eurytides* du groupe *protesilaus*; autrement dit les différences de dessin entre ces races sont plus grandes que les différences, spécifiques, entre les 11 espèces d'*Eurytides*. Pour illustrer ce phénomène nous mentionnons les races de *Lycaena virgaureae (miegii, osthelderi, chrysorhoas)*, *L. tityrus (acrion, bleusei)*, de *L. alciphron (gordius, bellieri)*, etc. [1]. Pourquoi ne considérons-nous pas ces races comme des espèces distinctes si leur dessin est si marquant? Quel dessin est spécifique et quel est racial ou aberrant? Il faudra donc montrer quelles sont les considérations qui dirigent nos recherches quand nous interprétons le dessin.

Le caractère spécifique du dessin s'établit justement seulement après l'examen de l'armature génitale. Si les genitalia chez plusieurs spécimens de *M. cinxia* sont identiques, ces spécimens appartiennent tous à cette espèce quoique certains aient de grandes taches noires et de petits espaces rougeâtres, les autres ont, par contre, de petites taches noires et les espaces rougeâtres sont très larges. Les caractères « grandes taches noires » et « larges espaces rougeâtres » sont, *ici*, des variations individuelles et non pas des caractères spécifiques. De même, nous procédons pour interpréter le dessin de certaines races géographiques très différentes l'une de l'autre. Quelque évidents que soient ces éléments du dessin, les formes dont il s'agit ne peuvent être considérées comme espèces distinctes mais seulement comme races géographiques si les genitalia sont identiques. Dans les deux cas, un élément de dessin quoiqu'il soit très prononcé, n'est pas un caractère spécifique mais une simple variation individuelle ou un caractère racial. Par contre, dans le cas des espèces au dessin convergent, un élément mineur du dessin peut être un caractère spécifique. Ainsi par exemple *E. protesilaus* diffère de *telesilaus* par la bande postmédiane [1] qui est bien développée, de forme triangulaire, tandis que chez *telesilaus* elle est réduite à une petite tache costale qui, très souvent, manque complètement [4]. Donc ici, un élément mineur — une petite tache costale — est un caractère spécifique. Nous sommes toujours arrivé à cette conclusion après l'examen de l'armature génitale. Si un spécimen présente le *processus dorsalis* dirigé dorso-ventralement et la harpe a l'extrémité distale étroite, il appartient à l'espèce. *E. protesilaus*, même si la bande post-médiane est réduite ou voire absente comme chez *E. telesilaus*.

Ces observations doivent attirer l'attention des tous les lépidoptéristes pour leur éviter des interprétations erronées qui conduisent aux erreurs taxonomiques. Un grand nombre de formes ou races géographiques ont été érigées à tort au rang spécifique par une interprétation erronée du dessin: *Iphiclides feisthameli*, *Pieris cheiranthi*, *P. bryoniae*, *Colias australis*, *Apatura metis*, *Vanessa vulcania*, *Lysandra coelestissima*, *L.albicans*, *L. caerulescens*, *L. arragonensis* et beaucoup d'autres « espèces ».

Le dessin des ailes doit être, bien entendu, pris en considération, mais interprété avec prudence et toujours comme un élément subsidiaire — après l'examen de l'armature génitale. Ce dernier examen est néces-

[1] Ce caractère, s'il était constant, pourrait très bien séparer les deux espèces, mais leur identification est très difficile puisqu'il est très variable.

saire même quand le dessin est très évident. Ainsi le dessin d'*antiopa* pourrait nous suggérer l'appartenance de cette espèce à un genre distinct de *Nymphalis* ; seulement l'armature génitale nous informe, avec précision, que *antiopa* appartient au genre *Nymphalis* et doit être placé à côté de *polychloros* en dépit du dessin si différent de ce dernier.

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# NOUVELLES ESPÈCES D'*ORIBATEI* (*ACARINA: ORIBATEI*)

PAR

N. VASILIU et MAGDA CĂLUGĂR

In this paper the authors describe the following three species new for science : *Oppia getica*, *Oppiella distincta* and *Urubambates romanicus*.
*Oppia getica* n.sp. is characterized by : long, reclinate interlamellar hairs ; very short lamellar hairs ; long pedicellated and spatulated sensillus with long branches ; cristae are absent.
*Oppiella distincta* n.sp. is characterized by : short and sharp rostrum with hairs inserted marginally ; pectinate sensillum with long branches ; costulae are in the prolongation of a dorso-sejugal acuminate carina.
*Urubambates romanicus* n.sp. is characterized by : elliptical body ; lamellae are narrowed in the middle ; small pteromorphes and epimeral hairs 1a and 1b are long and ciliary.

Parmi les Oribates du Nord de la Roumanie nous avons trouvé trois espèces nouvelles pour la science. Les résultats de nos études nous les publions dans la présente note.

## *SUPERFAMILLE OPPIOIDEA* Balogh, 1961

### Famille OPPIIDAE Grandjean, 1954

#### 1. *Oppia getica* n.sp.

*Diagnose*. Le rostrum allongé et pointu. Les costules courtes sont étroites au milieu et triangulaires à la base. Les poils interlamellaires très longs, fins et reclinés sont disposés sur la partie basale des costules. Au contraire, les poils lamellaires très courts sont insérés à la partie distale des costules. Les cristes sont absents.

*Dimensions* de l'idiosoma : longueur — 355 $\mu$ ; largeur 195 $\mu$.

*Morphologie*. Le prodorsum a le rostrum (ro) allongé et pointu. Les costules (co) courtes sont étroites au milieu et triangulaires à leur base. Parmi les quatre paires de poils du prodorsum, les lamellaires (la) disposées à l'extrémité distales des costules sont les plus courtes et les plus minces, tandis que les interlamellaires (il) insérées dans la portion basale de costules sont les plus longues et reclinées. On observe, avant de la bothridie (bo), la sous-lamelle (sla) avec un aspect de crête irrégulier. Au-dessus de la bothridie il y a le poil exobothrial (ex) qui est effilé et lisse. Le sensillus (ss) long, pédicellé et lancéolé porte des épines fines et longues.

Le notogaster arrondi et dépourvu de cristes, a neuf paires visibles de poils fins et lisses. Au-dessus des poils $r_3$ il y a la lyrifissure *im* (fig. 1).

Sur la face ventrale, les apodèmes (ap) sont conformes au type *Oppia*. Les poils épimériques (ép) petits et effilés sont disposés d'après

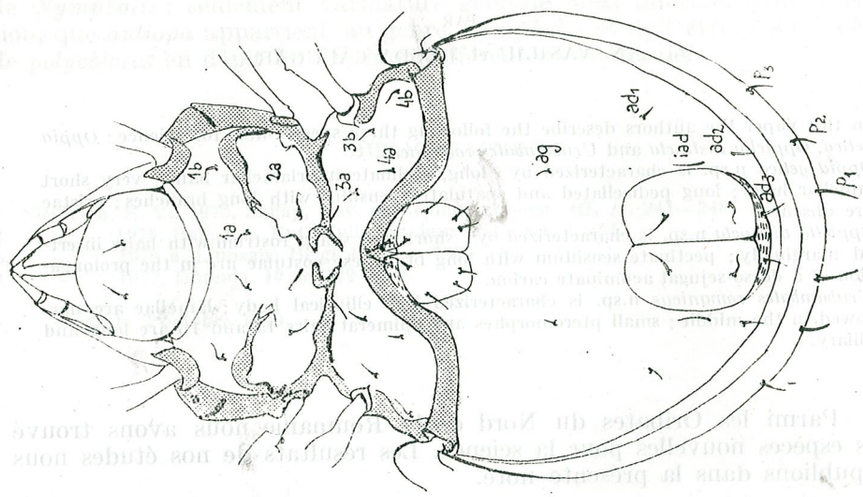


Fig. 2. — *Oppia gelica* n.sp. — corps, vue ventrale.

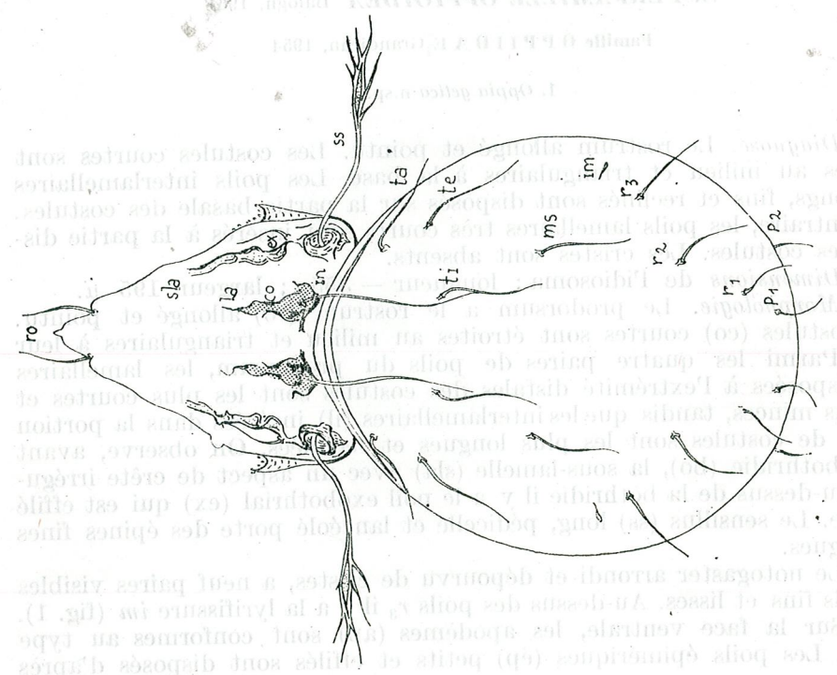


Fig. 1. — *Oppia gelica* n.sp. — corps, vue dorsale.



la formule : 2—1—2—2. Les volets génitaux trapézoïdaux et dépourvus d'un anneau périgénital ont cinq paires de petits poils effilés. Derrière les volets génitaux il y a une paire de poils aggénitaux (ag). Il y a, aussi, trois paires de poils adanaux (ad), dont la paire antérieure est la plus courte. Au voisinage des volets anaux sont disposées les lyrifissures—*iad* (fig. 2).

Les tarses sont tridactyles.

Holotype femelle et 12 paratypes chez les auteurs.

Terra-typica : forêt de hêtre Poieni, alt. 404 m (dép. de Iași). Biotope : litière. Date : 27. VIII. 1976.

### 2. *Oppiella distincta* n.sp.

*Diagnose*. Le rostrum court et pointu est bordé de poils rostraux. Les costules interrompues au milieu sont réunies par un sillon horizontal à peine visible. Le sensillus pectinat est arqué et dirigé en avant et vers le haut. Les poils lamellaires et interlamellaires sont disposés à l'extérieur des costules. La suture dorso-séjugale est acuminée.

*Dimensions* de l'idiosoma : longueur — 304 μ ; largeur — 174 μ.

*Morphologie*. Le prodorsum a le rostrum court et pointu. Les poils rostraux sont fixés en marge du rostrum. Les costules sont des sailliers interrompues au milieu où elles sont réunies par un sillon horizontal à peine visible. Les poils rostraux et exobothriaux égaux en dimensions sont plus longs que les lamellaires et interlamellaires, qui s'insèrent à l'extérieur des costules. Le sensillus pectinat a de longs cils sur un seul rang. Il est arqué et dirigé en haut. Les sous-lamelles ont l'aspect des baguettes chitineuses.

Le notogaster, piriforme, a la suture dorso-séjugale acuminée et bordée d'une carène fortement chitinisée disposée au-dessus des cristes. Cette carène se prolonge avec la partie basale des costules. On observe neuf paires de poils notogastraux minces et lisses. Les poils *ta* sont les plus courts. La lyrifissure *im*. est évidente (fig. 3).

Sur la face ventrale, les apodèmes sont bien développées. La région épimérale est ornée d'un fin réseau polygonal. Les poils épimériques petits et aiguisés sont disposés d'après la formule : 2—1—2—3. Les volets génitaux trapézoïdaux et dépourvus d'un anneau périgénital ont cinq paires de poils courts. En arrière des volets génitaux sont fixés une paire de poils aggénitaux. Les volets anaux entourés d'un anneau périanal portent deux paires de poils. Les trois paires de poils adanaux sont petits. La lyrifissure *iad* se trouve entre le poil $ad_2$ et le bord latéral de l'anneau périanal (fig. 4).

Les tarses sont tridactyles.

Holotype femelle et 26 paratypes chez les auteurs.

Terra-typica : Abieto-Piceetum, Gemenea, alt. 990 m (dép. de Suceava). Biotope litière. Date : 11. VII. 1979.

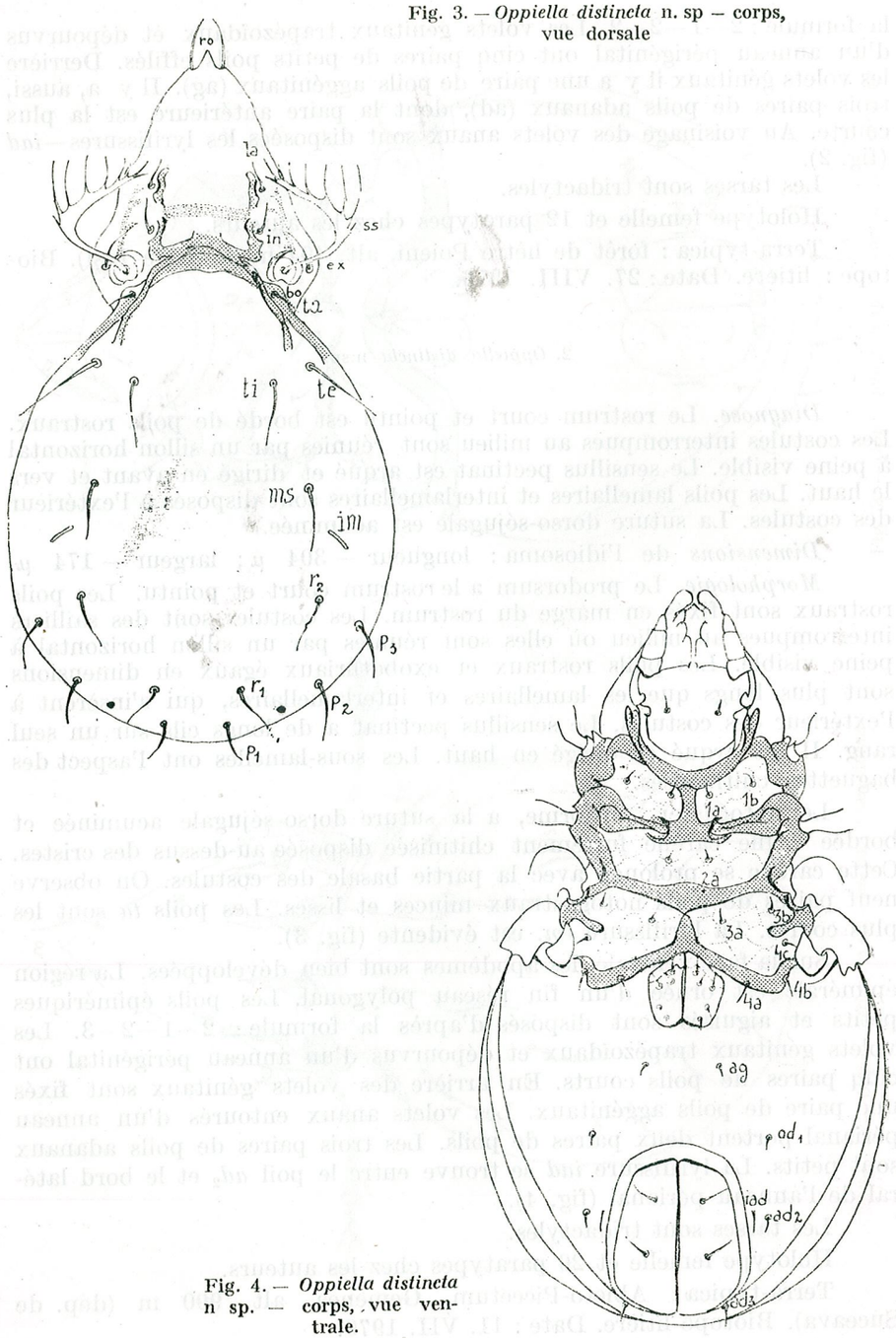


Fig. 3. — *Oppiella distincta* n. sp — corps, vue dorsale

Fig. 4. — *Oppiella distincta* n sp. — corps, vue ventrale.



## ***SUPERFAMILLE ORIBATULOIDEA*** Woolley, 1956

### Famille ORIBATULIDAE Thor, 1929

3. *Urubambates romanicus* n.sp.

*Diagnose*. Le corps très allongé est ellipsoïdal. Le rostrum arrondi est bien développé. Les poils interlamellaires sont longs, barbelés, divergents et orientés en avant. Le notogaster a des ptéromorphes étroites et allongées. Les poils épimériques 1*a*, 1*b* sont plus longs que les autres et barbelés.

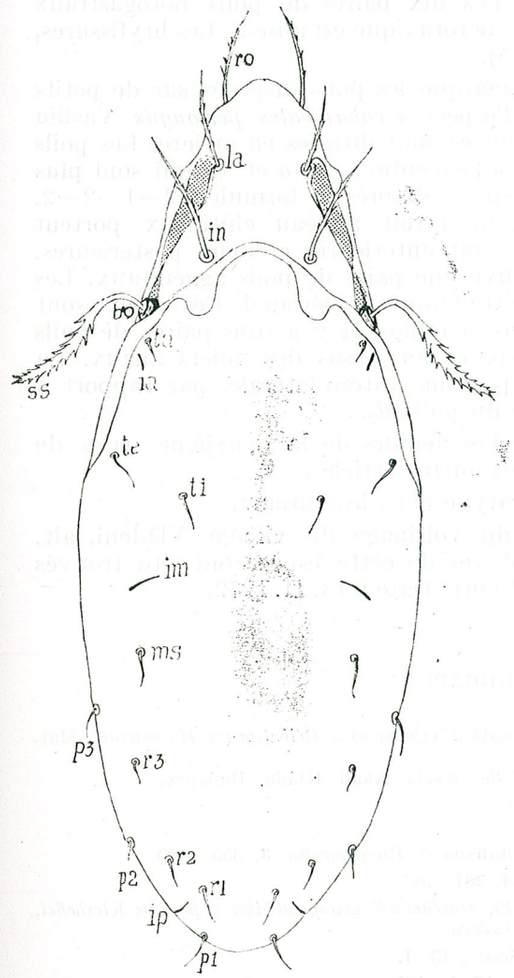


Fig. 5. — *Urubambates romanicus* n. sp. — corps, vue dorsale.

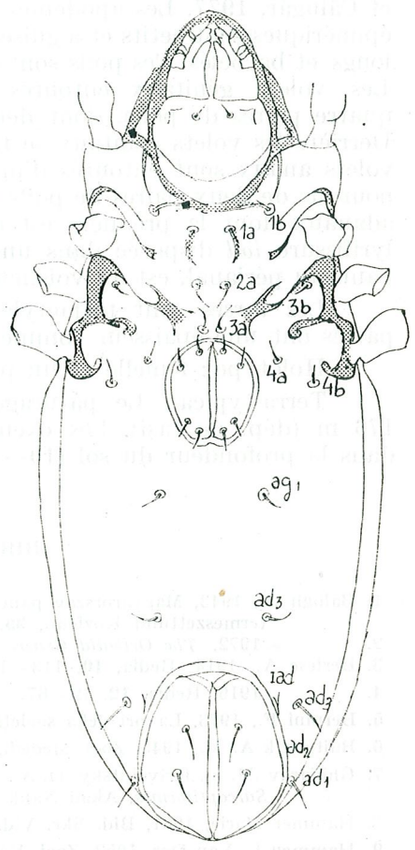


Fig. 6. — *Urubambates romanicus* n. sp. — corps, vue ventrale.

*Dimensions* de l'idiosoma : longueur — 415 μ, largeur 160 μ.

*Morphologie*. Le prodorsum a un rostrum bien développé et arrondi. Les poils rostraux sont minces, faiblement barbelés et convergents. Les lamelles disposées latéralement ont la partie distale arrondie, dépourvue de cuspis. Dans le tiers postérieur, elles sont étranglées. Les poils lamellaires effilés, peu barbelés et aussi convergents sont insérés à l'intérieur de la partie distale des lamelles. Les poils interlamellaires longs, effilés et barbelés sont divergents et orientés en avant. La bothridie est petite. Le sensillus claviforme-allongé a de fines épines.

Le notogaster, ellipsoïdal adhère bien au prodorsum. Son bord antérieur est très arqué, ainsi que la partie médiane qui avance jusqu'au niveau des poils interlamellaires. Les dix paires de poils notogastraux sont petits, lisses et effilés. L'organe octotaxique est absent. Les liryfissures, *ia*, *im*, *ip* sont bien visibles (fig. 5).

Sur la partie ventrale, on remarque les poils disposés sur de petits boucliers circulaires comme chez l'espèce *Urubambates perlongus* Vasiliu et Călugăr, 1977. Les apodèmes minces sont dirigées en arrière. Les poils épimériques sont petits et aiguisés, à l'exception de 1*a* et 1*b* qui sont plus longs et barbelés. Ces poils sont disposés d'après la formule : 2—1—2—2. Les volets génitaux entourés d'un étroit anneau chitineux portent quatre paires de poils, dont deux sont antérieures et deux postérieures. Derrière les volets génitaux se trouve une paire de poils aggénitaux. Les volets anaux sont entourés d'un étroit anneau périanal. Ces volets sont pourvus de deux paires de poils fins et effilés. Il y a trois paires de poils adanaux dont la première est disposée au-dessus des volets anaux. La lyrifissure *iad* disposée dans une position antéro-latérale, par rapport à l'anneau périanal, est au voisinage du poil $ad_2$.

Les tarses sont tridactyles. Les fémurs de la deuxième paire de pattes ont une épaisseur comme les autres articles.

Holotype : femelle et un paratype chez les auteurs.

Terra-typica : Le pâturage du voisinage du village Vlădeni, alt. 176 m (dép. de Iaşi). Les exemplaires de cette espèce ont été trouvés dans la profondeur du sol (10—12 cm). Date : 13. II. 1972.

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# ACTION OF FURADAN 75 ON THE TISSUES OF *NAUPHOETA CINEREA* (BLATTARIA–PANCHLORIDAE) AND *TANYMECUS DILATICOLLIS* GYILL. (COLEOPTERA–CURCULIONIDAE)

BY

MARIA TEODORESCU and VIORICA TRANDABURU

Tissue reactivity in *Tanymecus dilaticollis* and *Nauphoeta cinerea* was tested during *in vitro* conservation (2—24 hours) and under the influence of sublethal and lethal doses of Furadan 75. The most brutal alterations occurred in the corpora allata, the follicular epithelium and in the ovocytes, while little generalized damage was caused in the intestinal epithelium and the Malpighian tubes and even less so in the nervous and the endocrine tissues, where it is especially the nevroglia that are responding. The hypersensitivity of the follicular and nevroglial cells attests to their protective role for the ovocytes and the nervous cells, respectively. Furadan 75, even in a sublethal dose, affects the reproduction of insects.

The number of studies on the toxic action of chemical pollutants has been increasing. The toxicity of pesticides is transmitted to animals and gradually affects the human tissues, too. The treatments with insecticides diminish or suspend especially the prolificity of the insects disturbing the eclosion [2] — [6]. Such treatments should be necessarily preceded or accompanied by tissue tests on the degree of toxicity of the pesticides used. Therefore, we consider interesting to correlate our previous studies [8], [9] on the action of topically applied insecticides with the present investigation that placed the insect tissues into direct contact *in vitro* with the pesticide. Attempts to check the *in vitro* action of herbicides (2, 4, 5-T and simazine) were made also on the tissue of vertebrates (Gallus, Coturnix) [1].

## MATERIAL AND METHOD

Our studies involved the tissues of two insects : *Nauphoeta cinerea* and *Tanymecus dilaticollis*. The insects were fed and observed for one month under normal conditions in the laboratory. After that interval the following organs were collected from the females : the nervous tissue, the retrocerebral complex, the segmental perisympathetic organs, the genitalia, the intestine and the Malpighian tubes. Following their rapid collection, these organs were kept *in vitro* for 2—24 hours in a medium formed of the hemolymph of the respective insects and Furadan 75(0.05% and 0.1%). After 2 or 24 hours the experimental and control material was fixed in Bouin and stained with hemalaun-eosine.

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## RESULTS

*The genitalia*. We followed the morphology of germarium and vitellarium. Except for some contractions of the peripheral cells, the nutritive cells of the remaining germarium did not seem to be affected after two hours interval by the application of a low pesticide concentration. At the distal end of the germarium, the process of the ovarian follicles formation was blocked, follicular cells no longer grouping in a normal way. The nude ovocytes were crowded toward the vitellarium. The ovocytes nuclei showed no sign of alteration. The follicles already grouped in the vitellarium revealed the alteration of ovocytes and of follicular cells. The cells of the follicular epithelium become either hypertrophied or flattened, while the nucleus showed pycnosis. High pesticide doses induced degradation of the ovocyte cytoplasm by vacuolysis, the nucleus having the chromatin dislodged or agglutinated (Fig. 1).

*The Malpighian tubes*. The highly differentiated morphology of the Malpighian tubes epithelial cells, so very obvious during a normal activity, was seen *in vitro* only short time after a pesticide treatment in low doses. A stronger concentration led to the general flattening of the epithelial cells of the excretor tube, destroying them; some detached themselves and merged forming massive degraded cells in the excretor tube lumen (Figs. 2, 3).

*The intestinal epithelium*. Very few cells were affected by low Furadan concentrations; the cells of the remaining epithelium were tall and their nuclei preserved the fine chromatin normally distributed in the nucleoplasm. After 2 hours and particularly, after 24 hours, higher pesticide concentrations caused the degradation of epithelial nuclei, destroying the regenerative cell clusters and the shrinking of characteristic intestinal folds (Fig. 3).

*The nervous ganglions*. At 24 hours and even at 2 hours both concentrations of Furadan produced very slight alterations of the nervous tissue. Only a few neurons of the protocerebrum appeared to have their cytoplasm shrinked. In exchange, the perineuronal, periaxonal and perineuropilar glyocytes showed a pycnotic nucleus. The tracheolar system was unusually enlarged (Figs. 4, 5).

*The retrocerebral complex*. Corpora cardiaca and corpora allata responded differently to concentrations of 0.1%, especially after 24 hours (Figs. 6, 7). A generalized hypertrophy and vacuolysis of corpora cardiaca components autolysis of several glyocytes or only a shrinkage of their nuclei was noted. A massive degradation underwent also the periaxonal glyocytes of the nerves. The degraded cells of corpora allata were shrinked so that in several areas of the gland polymorphous masses produced by necrosed cells could be observed.

*The segmental perisympathetic organs*. As expected, these neurohemal organs responded similarly to corpora cardiaca (Figs. 8, 9, 10). The use of either Furadan concentration induced partial or total agglutination of the organ axis periaxonal glyocytes chromatin. Some peripheral epithelium glyocytes of the organ preserve their nucleus intact.

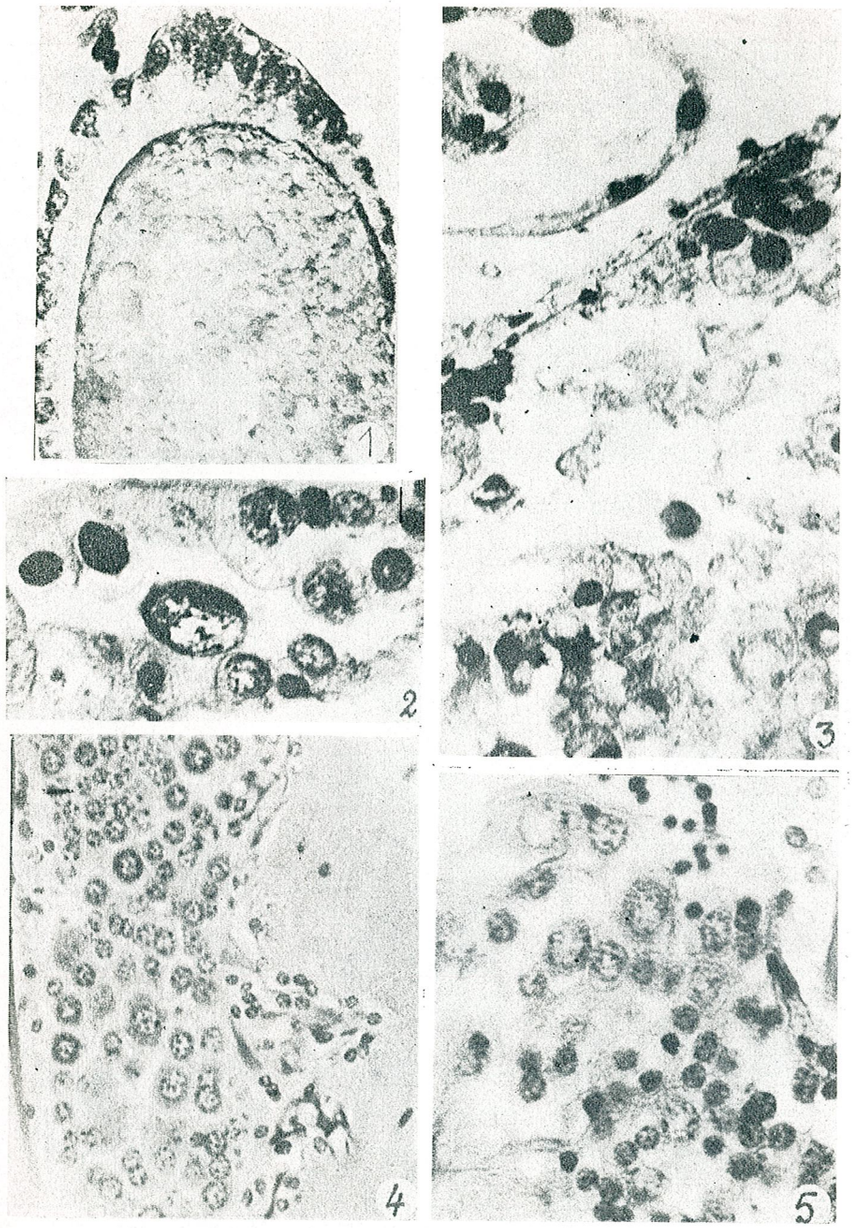
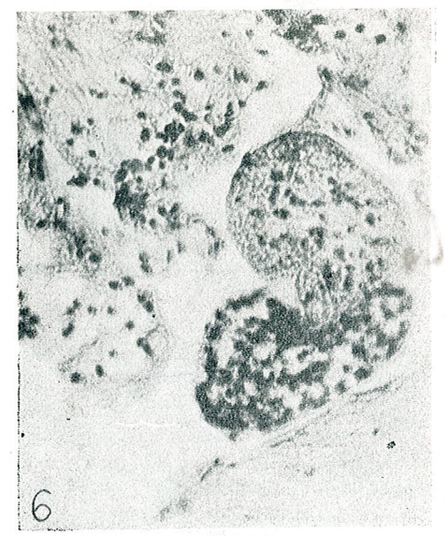
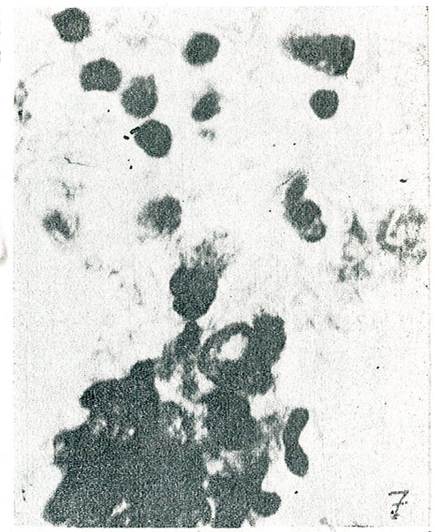
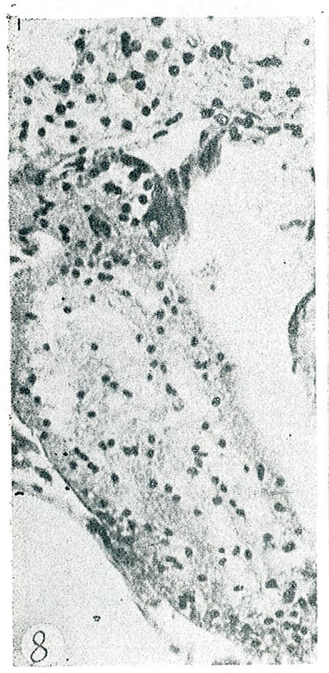
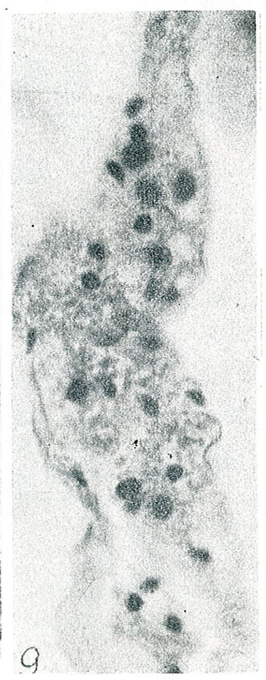


Fig. 1. — Ovarian follicle under 0.1 action. Ovocyte destroyed. The follicular cells begin flattening or hypertrophying.
Figs. 2, 3. — Malpighian tubes in normal conditions (Fig. 2) and under Furadan 0.1% influence (Fig. 3). Absorbant and regenerative epithelial intestine cells degradation after 24 hours with higher Furadan concentration (Fig. 3).
Figs. 4, 5. — Brain glyocytes reaction at 2 hours (Fig. 4) and 24 hours of action of 0.1% Furadan concentration (Fig. 5).



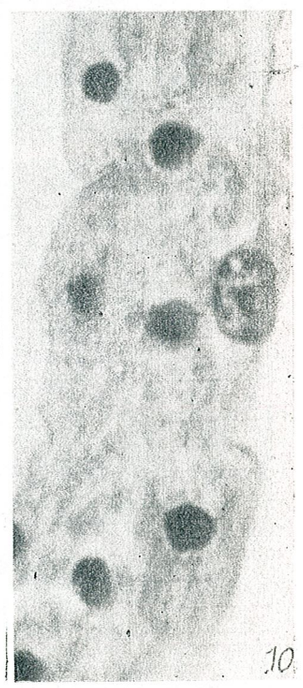




Fig. 6. — Shrinkage of corpora allata cells and pycnotic nuclei glyocytes cells of cardiaca nerves and corpora cardiaca cells (0.1% Furadan).

Fig. 7. — Intensive necrosis of corpora allata cells (0.1% Furadan).

Figs. 8, 9, 10. — Same massive glyocytes degradation in perisympathetic segmental nerves under 0.05% and 0.1% Furadan concentration.



## DISCUSSIONS AND CONCLUSIONS

Testing tissue response to the action of Furadan in *Tanymecus* and *Nauphoeta* supplements the studies on tissue sensitivity, especially the reproductive one, to the action of insecticides. Assessments should also be related to the period of moulting, when the ovocytes are more sensitive [7]. The *in vitro* testing of pesticides toxicity on the tissues provides conclusive results of the overall tissue response. In view of the extensive alterations induced by Furadan, the reproductive and the endocrine tissues were found to be the most sensitive ones followed by the Malpighian tubes, the intestine mucosa epithelium, the neurohaemal tissue and finally by the nervous tissue whose neuroglial elements are more degraded than the neurons. As previously shown [8, 9, 10] we wish to emphasize again the protective role of the covering epithelia, e.g. follicular and perineurial, which are affected by the insecticide toxicity. In addition, the alterations of the absorbant epithelium of the intestine and of the resorbant excreting one disturb the essential functions of the insect body.

The strong alterations of corpora allata would have degraded the reproductive tissue whose activity it controls, even if reproduction were less affected by pesticides. Our findings have revealed that Furadan acts similarly on corpora allata and the reproductive tissue. Testing even the minor effects of low Furadan doses has proved most conclusive in the case of the endocrine and especially of the reproductive tissues.

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# ULTRASTRUCTURAL MODIFICATIONS OF *CHALCIDES OCELLATUS* HEPATOCYTES AFTER DIBENZANTHRACENE ADMINISTRATION

BY

D. MIŞCALENCU, FLORICA MAILAT, M.EL ALFY and D. GEORGESCU

The administration of dibenzanthracene to lizard *Chalcides ocellatus* induces severe modifications in the hepatocytes of this animal. It can be admitted that these perturbations are induced by diol epoxides of dibenzanthracene which bind to different cell molecules. This way it forms molecular complexes which produce functional changes of cells such as massive destruction of mitochondria and the appearance of some rectilinear electronoclear structures which are supposed to appear from endoplasmic reticulum cisternae. Both formations (destructed mitochondria and electronoclear structures) became the lysis places of all altered materials in the cells.



## INTRODUCTION

The polycyclic aromatic hydrocarbons, like many other chemicals introduced in the organism, are modified in order to diminish their toxicity. Mainly, the disintoxication takes place in the liver which reacts with the carcinogenic complex molecules. Such studies have been subject for many authors [2], [5], [12], [14].

In recent years many new theories assert the transformation of polycyclic hydrocarbons in order to avoid their toxic effect, through the formation of diol epoxides either by aryl hydrocarbon hydroxylase [1], [13] or by cytochrome P-450 [3]. The present study intents to underline hepatocytes changes in the Egyptian lizard, *Chalcides ocellatus*, treated with dibenzanthracene.

## MATERIALS AND METHODS

*Chalcides ocellatus* (Reptilia, Squamata, Lacertilia, Scincinidae) individuals have been capturated from the Egyptian desert and kept in our laboratory. The animals received "per os" 10% dibenzanthracene (DBA), 0.2 $cm^3$ daily for 12 days. They have been sacrificed in November.

The small pieces of liver were double-fixed in glutaraldehyde and osmium tetraoxide, embbeded in vestopal and ultrasections were double-stained with uranyl acetate and lead citrate. The sections were examined by the Philips electronomicroscope.

## RESULTS

The action of DBA in stressed animals (because of the transport and captivity) induces severe perturbations in hepatic parenchyma cells.

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The nucleus of hepatocytes keeps its normal sizes but it can be observed the paucity of the chromatin which is peripherically situated. When the cytoplasm is invaded by electronoclear vesicles, the nucleus is smaller and peripherically situated. Its outline is slightly folded and displays incipient signs of pycnosis (Fig. 1). The mitochondria look very disturbed and in more advanced stages of destruction they become like some electronoclear vesicles which sometimes fuse one another and transform the whole cytoplasm in big vesicular cisternae (Figs. 1, 2).

The various aspect of modified mitochondria in the same cell (Fig. 2) indicates the destruction way which leads to the total electronoclarification. After the disappearance of cristae the whole content of the organelle is transformed in a granular mass among which electronoclear zones occur (Fig. 3). At the same time the altered mitochondrial membrane permits fusion of the matrix with the ambient cytoplasm.

In this stage of alteration in the matrix of mitochondria there can be seen huge electronodense granules which look like glycogen particles (Fig. 4). In some cells the rough endoplasmic reticulum is very poor and their cisternae surround the mitochondria (Figs. 2, 4). In these cells glycogen particles fuse one another and form compact masses (Figs. 2, 5). In other hepatocytes the rough endoplasmic reticulum has abundant small cisternae, the majority of which look like small vesicles (Fig. 6). In such hepatocytes the glycogen granules are almost absent (Fig. 6) and the mitochondria are not so abundant. These two types of hepatocytes exist in untreated animals too.

In the hepatocytes with compact masses of glycogen (Fig. 5) its lysis inside of electronoclear vesicles can be observed (Figs. 2, 3, 5). It seems that the electronoclear vesicles which form from mitochondria are those in which the lysis of glycogen occurs (Fig. 5); more, the fact that these vesicles have a double membrane proves their origin in mitochondria.

The rectilinear structures surrounded by an electronodense border (Figs. 1—6) or by compact zones of glycogen worth noticing. It seems that the role of these structures is to make the lysis of various cytoplasmic altered materials among which glycogen too (Fig. 4). Sometimes these rectilinear structures fuse with electronoclear vesicles (Fig. 6). It can be observed that electronodense masses which surround these structures can be seen inside of their electronoclear lumen. Maybe these formations are cisternae of the endoplasmic reticulum which together with the transformed mitochondria assume their main function of lysis.

## DISCUSSIONS

We consider that the changes of hepatocytes in *Chalcides ocellatus* treated with DBA represent an effect of reaction between diol epoxide of DBA and the molecules of cellular constituents. Even that diol epoxide of DBA appears either by aryl hydrocarbon hydroxylase [1], [13] or by hemoprotein cytochrome P-450 [3] implication its great reactivity with the

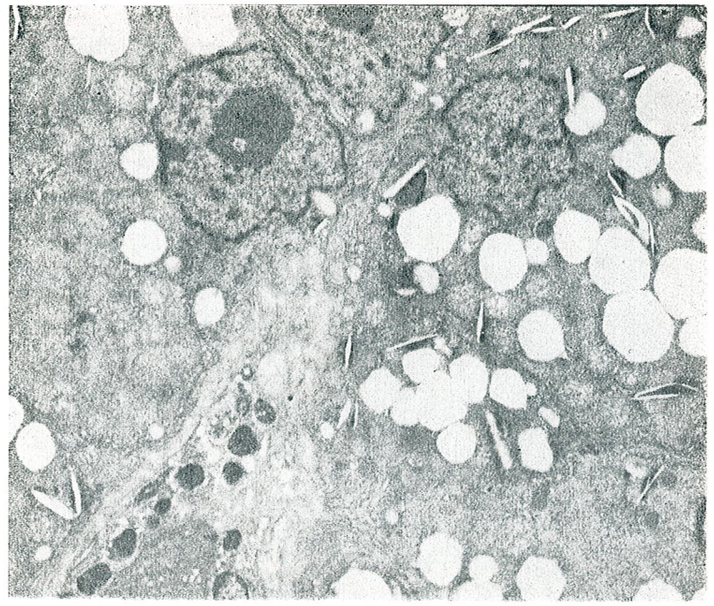

Fig. 1. — Modificated hepatocytes in *Chalcides ocellatus* treated with dibenzanthracene. The nucleus displays chromatine paucity and the cytoplasm contains electronoclear vesicles which are altered mitochondria; these are transformed in deposits for degraded materials like the rectilinear electronoclear structures probably derived from endoplasmic reticulum cisternae.

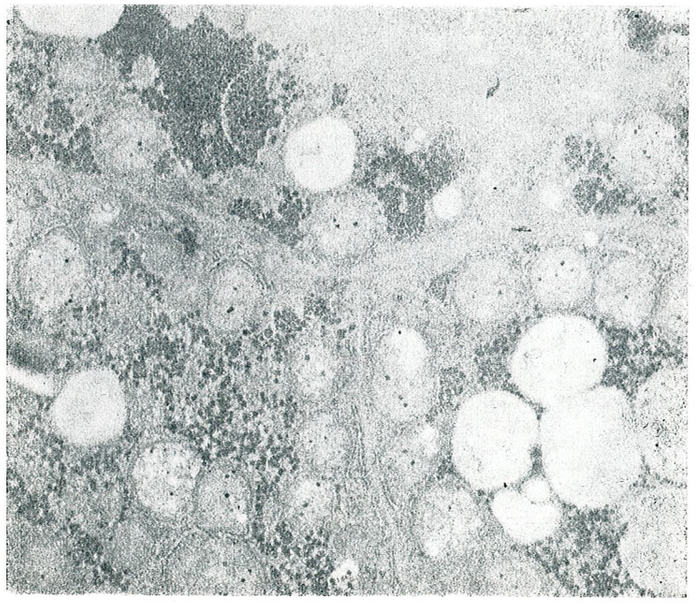

Fig. 2. — Various stages of the altered mitochondria and characteristically condensation of glycogen particles.

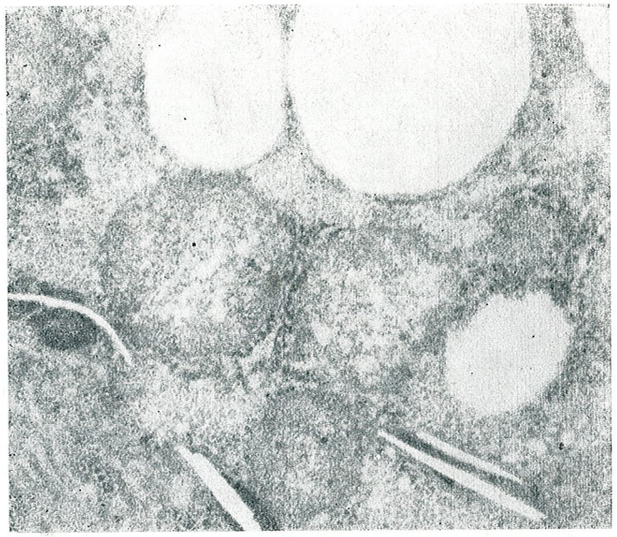

Fig. 3. — Lysis of electronoclear vesicles in which mitochondrial membranes can be seen, here and there. This cell illustrates long electronoclear formations ssurrounded by electronodense masses under degradation.

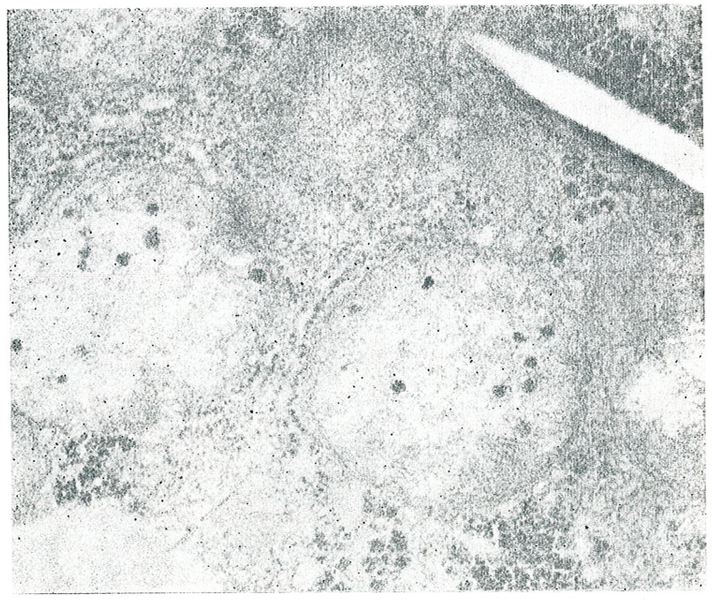

Fig. 4. — Glycogen particles in mitochondria. On the side of the rectilinear electronoclear structures glycogen particles in lysis can be seen.

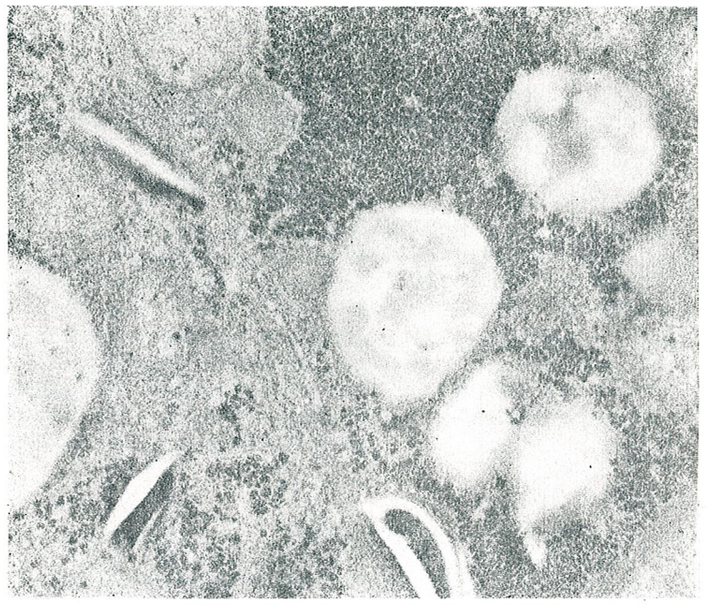

Fig. 5. — Compact masses of glycegen-a characteristic aspect for *Chalcides ocellatus* hepatocytes. The glycogen particles are degraded inside of spherical vesicles.

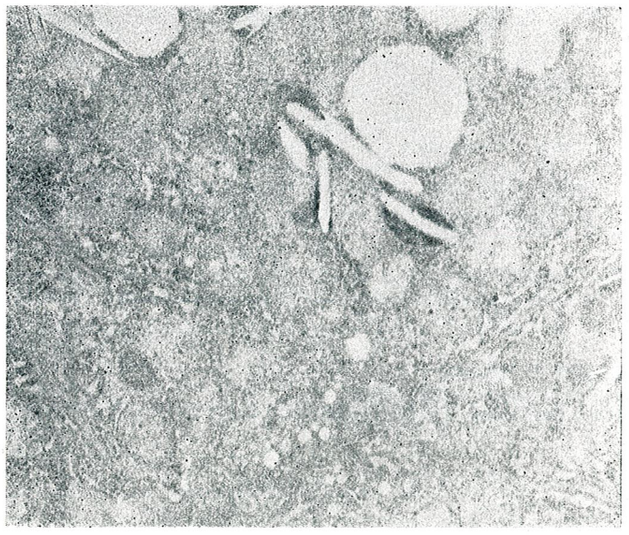

Fig. 6. — Hepatocytes with inconspicuous amounts of glycogen. Endoplasmic reticulum displays small vesicles. Electronoclear vesicles fuse with rectilinear electronoclear structures.

molecules of cellular constituents seems to be given by the "bay" region [3] and not by the "K" region of hydrocarbons.

Some authors suppose that microsomal cytochrome P-450 plays an important role in the transformation of polycyclic hydrocarbons which —by their keto- and hydroxyl-groups — are binding to the plan of cytochrome molecule, according to the model of interaction of a steroid with the heme of cytochrome P-450 (Estabrook et al. — in Gelboin et al. [3]).

There are general considerations that the resulting metabolites accumulate in the mitochondria and microsomes [4]. In *Chalcides ocellatus* liver they accumulate in the mitochondria but also in some rectilinear structures which we suppose that derived from endoplasmic reticulum cisternae. These structures become lysis structures which destroy all altered organelles (mitochondria, microsomes, glycogen, etc.) and other substances degraded by DBA diol epoxide. The strongest altered organelles are the mitochondria probably because the DBA molecules interact with the few cytochromes of their membrane and in this way the membranes are disorganized.

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# EFFECTS OF EXPOSURE TO HIGH TEMPERATURE UPON GLYCOGEN CONTENT IN THE POSTERIOR ADDUCTOR MUSCLE AND THE HEPATOPANCREAS OF *MYTILUS GALLOPROVINCIALIS* (L) OF THE BLACK SEA

BY

NINA ŞILDAN, IOSIF MADAR and EUGEN A. PORA *

The effects of exposure to temperatures above the ambient upon the glycogen content in the posterior adductor muscle and the hepatopancreas of *Mytilus galloprovincialis* were studied.

It has been established that 60 hours exposure at 25°C did not alter the glycogen stores in the muscle and hepatopancreas as compared to the control group (held for 60 hrs at 21.5°C).

Keeping mussels for 15, 30 and 60 hours at 30°C resulted in a rapid and significant decrease of the glycogen content of both tissues as against control values. Percentage glycogen loss from muscle and hepatopancreas was approximately the same and increased markedly and proportionally to the time of exposure.

Glycogen amounts can be extremely high in well fed marine bivalves, usually varying between 5 and 40 percent of the dry body weight, with extremes lying around 60 percents. On the basis of the fact that all tissues of the body are involved in the storage of glycogen, it was stated that bivalves have a predominantly glycogen oriented metabolism [5].

In a previous paper [7], we reported that the exposure of *Mytilus galloprovincialis* to temperatures above the ambient resulted in a significant decrease of the circulating glucose concentration. On the other hand, it was shown that the relative long lasting temperature and the nutritive stress led to a decline in the body condition of *Mytilus edulis* due to the loss of both carbohydrate and protein reserves in tissues [4], [12]. In this respect, it has been established that, in *Mytilus edulis* and *Crassostrea virginica*, the effects of temperature stress varied seasonally [4], [9]. Thus, in the seasons of the year, when the glycogen stores in the body tissues were at maximum, the thermal stress resulted in the utilization of carbohydrates. On the contrary, when the glycogen reserves were low, the proteins served as the main source of energy.

Taking into consideration the above findings, in the present work we summarized the effects of short-term exposure to temperatures above the ambient upon the glycogen content in the posterior adductor muscle and the hepatopancreas of *Mytilus galloprovincialis*.

## MATERIAL AND METHODS

For all the experiments, carried out between 1—10 August 1976, mussels were collected from the low-water (0.5 m) littoral zone of the Romanian shore of the Black Sea near Agigea (I.R.C.M. —Constanţa).

* The technical assistance of Magdolna Anghelea is acknowledged

The mean ambient (field) temperature was of 21.5°C, the mean salinity of 17.7 ‰ and the oxygen content of 118 ‰.

Approximately 200 individuals of 4.6 ± 0.2 cm length, with comparable body weights, were selected. After cleaning the shells, the mussels were kept for accomodation in natural, sand-filtered, aerated, sea water at ambient temperature (21.5 ± 0.5°C). After 24 hours the animals were divided at random into five groups of 30—50 individuals and placed in aquariums at a density of 1 mussel per 1 kg sea water. The experimental thermal environments were controlled to ± 0.5°C; the photoperiod was maintained at 14 hrs light and 10 hrs darkness; the water from the aquariums was changed twice a day. Throughout the experiments the following groups of *Mytilus galloprovincialis* were used :

— Group I, control mussels kept at ambient temperature (21.5°C) for 60 hours;
— Group II, mussels kept at 25°C for 60 hours;
— Group III, mussels kept at 30°C for 15 hours;
— Group IV, mussels kept at 30°C for 30 hours;
— Group V, mussels kept at 30°C for 60 hours.

In order to obtain comparable results, since the mussels were not fed throughout the experiments, the animals belonging to groups III and IV wer emaintained at ambient temperature for 45 hours, respectively for 30 hours before placing them at 30°C. Thus, the total time spent in laboratory was the same (24 hrs accomodation interval + 60 hrs) for each experimental group.

At the end of all experiments, the shells were opened, by cutting unilaterally the posterior adductor muscle, and the soft body of the mussels was carefully blotted with filter paper. Small pieces of muscle and hepatopancreas (about 20—30 mg) were quickly removed for glycogen analysis. The glycogen determination was made according to R. Montgomery [8]. All analyses were carried out in duplicate or triplicate and the mean values were used to calculate the average glycogen content of each tissue.

The mussels were considered dead and discarded, if the mantle muscular border failed to contract when stroked with a glass rod.

The data, expressed as mg glycogen per 100 g fresh tissue weight, were compared according to Student's $t$ test, the differences being considered statistically significant when $P < 0.05$.

## RESULTS AND DISCUSSIONS

Table 1, and figures 1 and 2, summarize the average glycogen content in the posterior adductor muscle and hepatopancreas of *Mytilus galloprovincialis* kept at ambient and above ambient temperatures as well as the percentage modifications as compared to the control values.

Elsewhere, we pointed out [6] that circulating glucose levels in the hemolymph of *Mytilus galloprovincialis* during summer are low, as compared to glycogen stores [10], ranging from 3.7 to 30.6 mg % depending on the body size (length in cm). Nevertheless, the blood glucose concentration, as well as the glycogen content of tissues, is relatively constant within well-defined size groups of mussels.



*Table 1*

Temperature and exposure period dependent changes of mean glycogen content ± ES in the muscle and hepatopancreas of *Mytilus galloprovincialis*

| | mg glycogen/100 g wet tissue weight | | | | |
|---|---|---|---|---|---|
| | 21.5°C | 25°C | 30° C | 30°C | 30°C |
| | I<br>60 hrs | II<br>60 hrs | III<br>15 hrs | IV<br>30 hrs | V<br>60 hrs |
| Muscle | 3875±198<br>(16) | 3483±192<br>(16) | 3138±189<br>(16) | 2173±126<br>(16) | 1508±163<br>(20) |
| | —<br>— | −10.11 %<br>$P>0.05$ | −19.02 %<br>$P<0.02$ | −43.92 %<br>$P<0.001$ | −61.08 %<br>$P<0.001$ |
| Hepatopancreas | 7966±380<br>(16) | 6916±548<br>(16) | 6027±336<br>(15) | 4814±425<br>(16) | 3473±428<br>(20) |
| | —<br>— | −13.18 %<br>$P>0.05$ | −24.34 %<br>$P<0.001$ | −39.57 %<br>$P<0.001$ | −56.40 %<br>$P<0.001$ |

The number of animals is given in parantheses. Least significant percentage differences as compared to the control group I (kept at ambient temperature) are considered at $P<0.05$.

B.L. Bayne [1] calculated that in *Mytilus edulis* the free sugar pool can meet the metabolic energy demands for 15.5 to 190 minutes, depending on the season. Thus, under stress conditions, the energy metabolism of mussels must be greatly reliant on the selective utilization of various energy stores. As mentioned before, it is generally accepted that, in summer, carbohydrates are the main source of energy, both in aerobic and anaerobic conditions. In this connection, it can be specified that in *Mytilus edulis* the great majority of stored carbohydrates is present under the form of glycogen.

In a previous work [7] we found that exposure of *Mytilus galloprovincialis* to 25°C for 60 hours resulted in a marked decrease (with 58.62 %) of the blood glucose level. The results of the present research show that the mussels held 60 hours at 25°C maintain their glycogen amounts, in muscle and hepatopancreas, close to the values found in control mussels held at ambient temperature. These data strongly suggest that the experimental temperature of 25°C is situated within the thermal tolerance zone [2] of this species, and that the slightly enhanced metabolic demands of animals can be compensated by the increased utilization of circulating glucose [7].

When mussels are kept in a thermal environment of 30°C the glycogen stores, from muscle and hepatopancreas, are markedly affected. Thus, after a 15 hours lasting exposure to this temperature the glycogen amounts are significantly diminished, with 19.02 % ($P < 0.02$) in the

posterior adductor muscle and with 24.34% ($P < 0.001$) in the hepatopancreas, as compared to the control values. However, the loss in glycogen reserves is not accompanied by noticeable changes in the blood glucose content [7] suggesting a physiological adaptative response during which the homeostatic control of blood glucose level is preserved. In

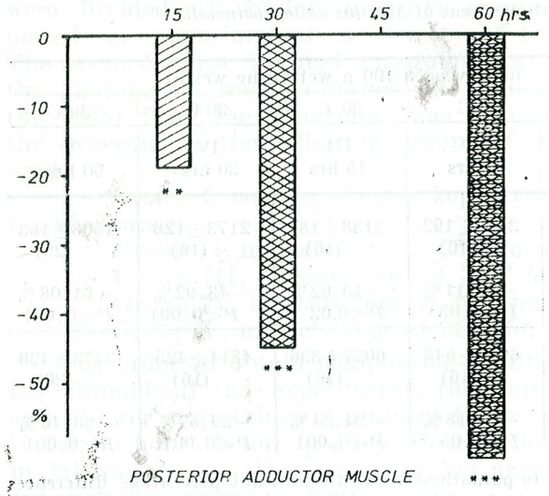


Fig. 1. — Percentage decrease of the glycogen content in the posterior adductor muscle after 15, 30 and 60 hours exposure of *Mytilus galloprovincialis* at 30°C as compared to the control values (21.5°C).

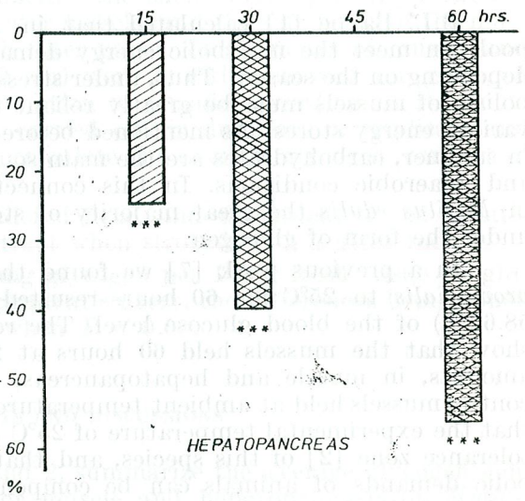


Fig. 2. — Percentage decrease of the glycogen content in the hepatopancreas after 15, 30 and 60 hours exposure of *Mytilus galloprovincialis* at 30°C as compared to the control values (21.5°C).

this respect, studying the physiological adaptation of *Mytilus edulis* to cyclic temperatures, J. Widdows [13] supposed that adaptative responses to upper thermal limits could be stimulated or induced by a brief exposure to the maximum temperature experienced.



Extension of the exposure time at 30°C, to 30 hours and 60 hours, leads to dramatic changes in the glycogen content of both tissues examined. Thus, the glycogen used during 30 hrs and 60 hrs is about 43.92 % ($P < 0.001$) respectively about 61.08% ($P < 0.001$) of the glycogen stored in the muscle (Fig. 1). As concerning the hepatopancreas the glycogen consumption, under these conditions, is similar to that found in the muscle, the losses averaging 39.57% ($P < 0.001$) after 30 hours and 56.40% ($P < 0.001$) after 60 hours spent at 30°C as against control values (Fig. 2).

The breakdown of glycogen reserves is accompanied by a sharp drop in the blood glucose level (with 58.87%) after 30 hours maintenance at 30°C. This marked decrease of glucose content is only slightly accentuated (— 64.37%) in the case of mussels exposed for 60 hours at 30°C.

Heat tolerance studies on *Mytilus galloprovincialis* [10] showed that no mortalities were recorded within 15 hours exposure to 30°C, while in animals subjected to this temperature for 30 and 60 hours the mortality averaged 10% and 50% respectively. The high mortality curves registered at 60 hours exposure periods certify the fact that the animals are severely stressed.

All these data suggest that the temperature of 30°C is situated beyond the limits of the thermal tolerance zone of *Mytilus galloprovincialis* and that at this temperature the mussels fail to compensate their high energy requirements and to survive indefinitely.

It is accepted that in mussels the anaerobic metabolism is entirely reliant on carbohydrates. Under anaerobic conditions, A.D. Zwaan and D.I. Zandee [14] found in *Mytilus edulis*, at 20°C after a 48 hours period, a 23% increase in the utilization of glycogen from the muscle and hepatopancreas (the Pasteur effect) as reported to the control values. Taking into consideration the decreased oxygen tension in the thermal environment of 30°C, the drastic glycogen breakdown may be partially ascribed to the reduction of oxygen.

Thus, our results lead to the supposition that *Mytilus galloprovincialis* survives an exposure to a high temperature of 30°C by producing energy, both aerobically and anaerobically, from the breakdown of glycogen stores and that glycogen consumption is proportional to the time of thermal and hypoxic stress.

## CONCLUSIONS

1. The exposure of *Mytilus galloprovincialis* at 25°C for 60 hours does not affect the glycogen content of the muscle and hepatopancreas.

2. In mussels subjected to a temperature of 30°C a significant glycogen breakdown is noticed, both in the muscle and the hepatopancreas, after 15, 30 and 60 hours exposure periods. The glycogen consumption is proportional to the time of thermal stress suggesting that *Mytilus galloprovincialis* survives high temperatures by producing energy from glycogen reserves.

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# EFFECTS OF ANTIBIOTICS ON THE CHICK EMBRYO DEVELOPMENT

BY

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In 500 hen eggs the effect of the following antibiotics was tested : Ampicillin, Oxacillin, Kanamycin, Streptomycin, Gentamycin, Chloramphenicol hemisuccinate, Solvocillin and Novobiocin. The antibiotics were introduced in the 72nd hour of incubation. The results show an embryotoxic effect for : Solvocillin, Chloramphenicol, Novobiocin and Oxacillin which induced a high mortality rate (80%, 71%, 65% and 60% respectively). In addition the following malformations were noticed :exencephaly, anophthalmy, meningocelle, distorted legs, asymmetrical beak, eviscerations, in the eggs treated with : Chloramphenicol, Novobiocin, Kanamycin and Oxacillin. No malformations occurred in the lot treated with Ampicillin.

Antibiotics, drugs that have been succesfully used in therapy for over 40 years may also have some adverse reactions such as : ototoxicity, nephrotoxicity, medullotoxicity and teratogenic effect [1].

As concerns the teratogenic effect, only tetracyclines, aminoglycosides and tuberculostatics are implied. Experiments carried out on various animals have showed that the list of these antibiotics should be enlarged and that other drugs in this group should be used with great caution during pregnancy [2] — [5], [7] — [10].

## MATERIAL AND METHOD

Five hundred Rock hen eggs from the Săftica farm were used to test the effects of : Ampicillin (0.5 mg/egg), Oxacillin (0.5 mg), Streptomycin (0.5 mg) Gentamycin (0.16 mg), Kanamycin (0.5 mg), Novobiocin (0.5 mg), Solvocillin (0.5 mg) and Chloramphenicol hemisuccinate (0.5 mg/egg).

The eggs were incubated at 37°C in 60% environmental humidity. After 36 hrs, small windows were cut in the calcareous shell of the eggs in order to apply the drugs and observe embryo development.

The antibiotics were dissolved in Tyrode solution pH 7.4 and injected after 72 hrs of incubation in doses of 0.1 ml solution, to the extra-embryonary area. The embryos were daily observed and at the end of the incubation period sacrificed.

## RESULTS AND DISCUSSIONS

### 1. SURVIVAL OF THE EMBRYOS

Good survival rate was recorded with the lots treated with Gentamycin (90%), Ampicillin (86%), Kanamycin (81%) and Streptomycin (77%). Survival in the control lot was 95%.

Death occurred among embryos on the 7th day in the control and ampicillin lots and on the 5th and 18th in the gentamycine lot. Streptomycin caused death on the 5th, 7th and 12th day and Kanamycin on the 15th day.

Musy [11] noticed that under artificial incubation conditions chick embryos may reach a mortality rate of up to 30 %. Working with 700 eggs he noticed that there are two critical periods in which chick embryo mortality is high : on the 5th and the 18th day.

In the lots treated by us with Oxacillin, Chloramphenicol, Novobiocin and Solvocillin the survival is much decreased : 40 %, 26 %, 35 %

*Table 1*

Survival percentage of chick embryos treated with antibiotics in stage 20 of development

| Days of incubation | Antibiotics | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Ampicillin | Oxacillin | Streptomycin | Kanamycin | Gentamycin | Novobiocin | Cloramphenicol | Solvocillin |
| | % | % | % | % | % | % | % | % | % |
| 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 4 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 5 | 100 | 100 | 93 | 100 | 95 | 95 | 92 | 100 | 93 |
| 6 | 100 | 100 | 93 | 100 | 95 | 95 | 85 | 100 | 93 |
| 7 | 95 | 86 | 88 | 95 | 86 | 95 | 85 | 100 | 86 |
| 8 | 95 | 86 | 88 | 90 | 86 | 95 | 85 | 93 | 80 |
| 9 | 95 | 86 | 88 | 90 | 86 | 95 | 85 | 93 | 73 |
| 10 | 95 | 86 | 88 | 90 | 86 | 95 | 85 | 86 | 73 |
| 11 | 95 | 86 | 88 | 90 | 86 | 95 | 78 | 80 | 66 |
| 12 | 95 | 86 | 80 | 81 | 86 | 95 | 64 | 80 | 46 |
| 13 | 95 | 86 | 73 | 81 | 86 | 95 | 64 | 66 | 40 |
| 14 | 95 | 86 | 66 | 81 | 86 | 95 | 64 | 53 | 40 |
| 15 | 95 | 86 | 40 | 81 | 81 | 95 | 57 | 40 | 33 |
| 16 | 95 | 86 | 40 | 81 | 81 | 95 | 35 | 40 | 20 |
| 17 | 95 | 86 | 40 | 81 | 81 | 95 | 35 | 40 | 20 |
| 18 | 95 | 86 | 40 | 81 | 81 | 90 | 35 | 26 | 20 |
| 19 | 95 | 86 | 40 | 77 | 81 | 90 | 35 | 26 | 20 |
| 20 | 95 | 86 | 40 | 77 | 81 | 90 | 35 | 26 | 20 |
| 21 | 95 | 86 | 40 | 77 | 81 | 90 | 35 | 26 | 20 |

and 20 % respectively. Our results are of statistical significance ($P < 0.01$). The death of embryos was recorded throughout the incubation period (Table 1).

Fig. 1 displays the percentage of chick embryos survival in eight experimental lots. Embryotoxicity of these antibiotics can be appreciated through the precentage of induced lethality. The most toxic were the following : Solvocillin (80 % embryonary death), Chloramphenicol (74 %), Novobiocin (65 %) and Oxacillin (60 %). Gentamycin, Ampicillin, Kanamycin and Streptomycin proved to be less toxic.

## 2. INDUCTION OF MALFORMATIONS

In the Ampicillin lot no significant malformations were noticed. The Oxacillin lot showed 15 % malformed chickens : meningocele, micro-



cephalus, distorted fingers. Kanamycin induced malformations in 20 % of the chickens : head asymmetry, microcephalus, distorted legs and fingers, eviscerations. Streptomycin and Gentamycin did not cause significant malformations. However, some of the chickens presented asymme-

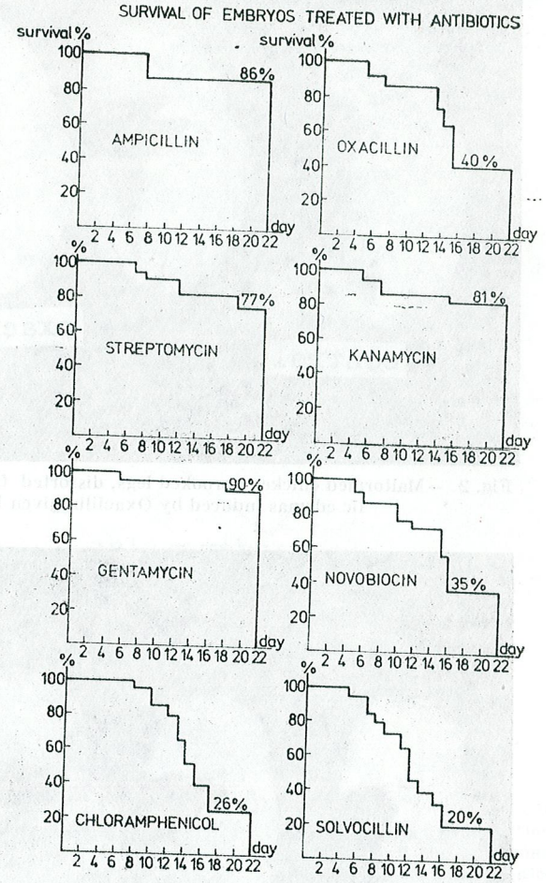


**Fig. 1. — Survival of embryos treated with antibiotics.**

tric body and distorted fingers. Chloramphenicol induced malformations in 50 % of the embryos. There was one case of exencephaly with lack of parietal and frontal bones ; other malformations, such as anophthalmia, distorted beak, microcephaly, distorted legs and fingers and eviscerations were also noticed. Solvocillin induced 15 % malformed embryos : head asymmetry, distorted beak, distorted fingers. Novobiocin caused 20 % of the chickens to be malformed : beak and head asymmetry, microcephaly, abnormal legs (see Figs. 2, 3, 4, 5).

Table 2 presents the types of malformations induced by the antibiotics examined by us.

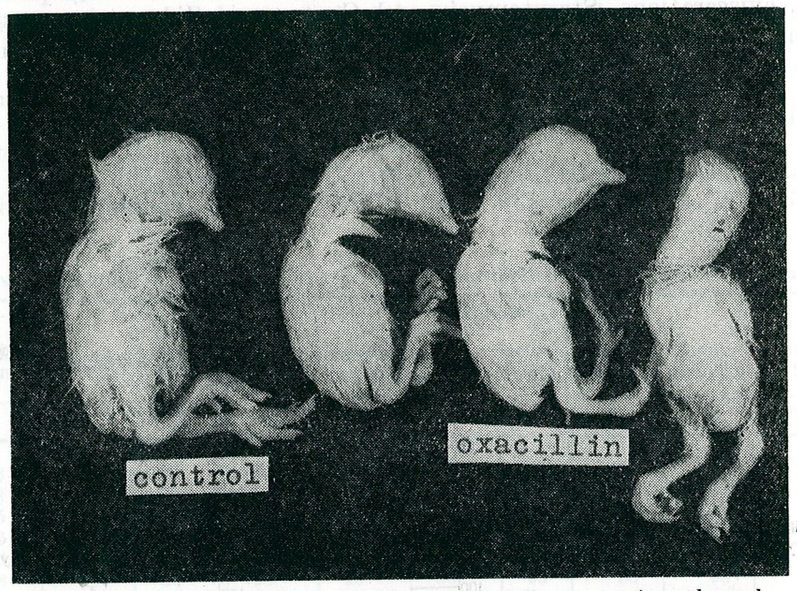


Fig. 2. — Malformed chicken ; crooked legs, distorted fingers, meningocele, cephalic edemas induced by Oxacillin given in stage 20.

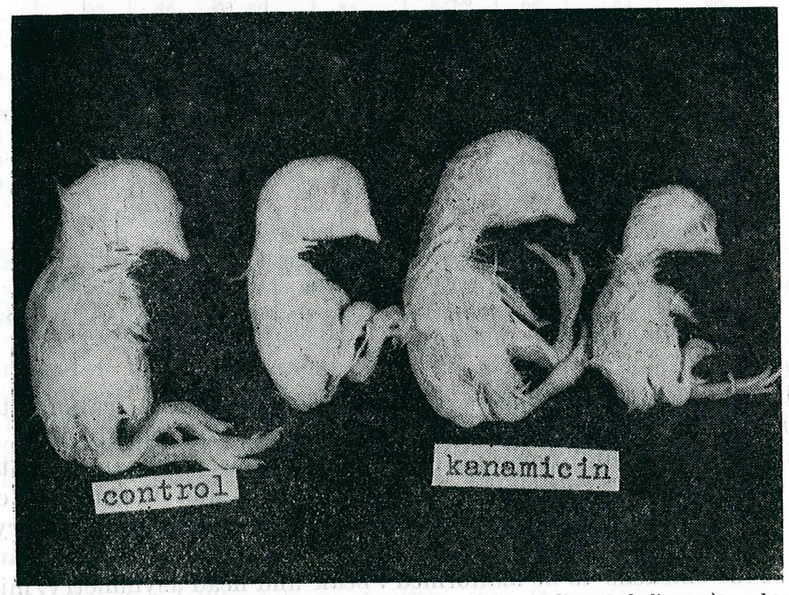


Fig. 3. — Underdeveloped chicken (cephalic edemas, distorted fingers) under the action of Kanamycin.



Musy [11] shows that artificial incubation may yield 4% malformed embryos. In our experiment the control lot (55 embryos) showed no malformation.

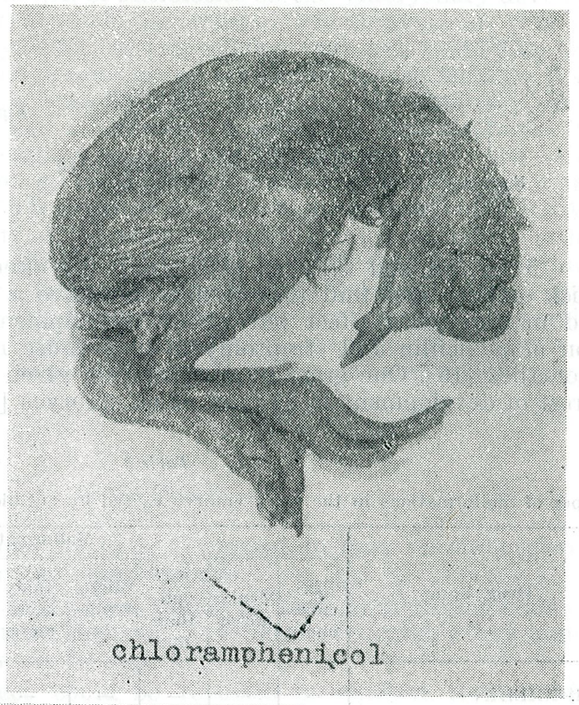


Fig. 4. — Malformed chicken : exencephaly, anophthalmy, abnormal beak, caused by chloramphenicol.

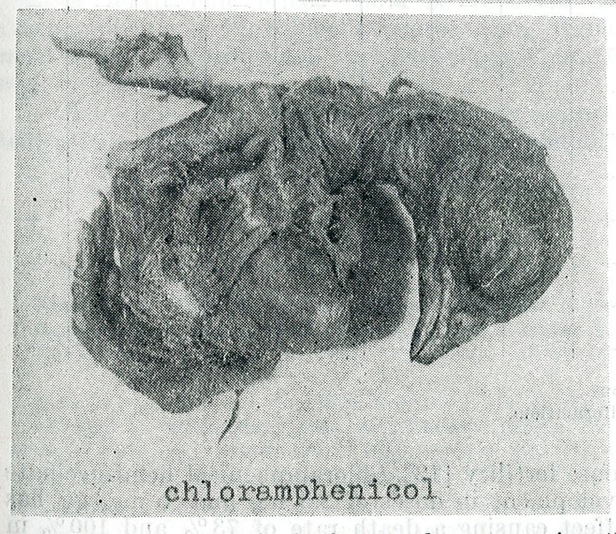


Fig. 5. — Malformed chicken with eviscerations, malformed legs and wings, caused by chloramphenicol.

There are relatively few works concerning the teratogenic effect of antibiotics. Fillipi (cited by [13]) reports one case of abortion in a wom-

an treated with penicillin during pregnancy. Doses of 0.5 mg penicillin /egg given in stage 10 of chick embryo development determine a growth inhibition of 41% [14], [6].

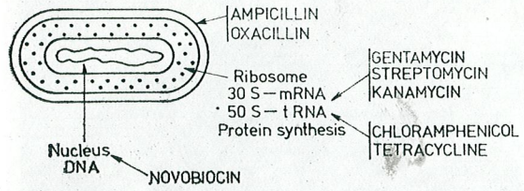


Fig. 6. — Mechanism of action of antibiotics.

Trăistaru and Cilievici [16] study the effect of Ampicillin on the chick embryo and find that it does not have a significant embryotoxic and malformative effect but causes developmental deficiency. Association of penicillin and streptomycin may cause micromyelia and digital anomalies [13]. One gram of streptomycin given in stage 10 causes the arrest of development in 31% of chick embryos [14]. Kanamycin causes

*Table 2*

Types of malformations in the chick embryo caused by antibiotics

| Drug dose | Malformations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exencephaly | Meningocele | Anophthalmia | Microcephaly | Body asymmetry | Head asymmetry | Crooked legs | Distorted fingers | Evisceration |
| AMPICILLIN 0.5mg | — | — | — | — | — | — | — | — | — |
| OXACILLIN 0.5mg | — | + | — | + | — | — | — | + | — |
| KANAMYCIN 0.5mg | — | — | — | + | — | + | + | + | + |
| STREPTOMYCIN 0.5mg | — | — | — | — | — | + | — | + | — |
| GENTAMYCIN 0.16mg | — | — | — | — | — | + | — | — | — |
| CHLORAMPHENICOL 0.5mg | + | — | + | + | + | + | + | + | + |
| SOLVOCILLIN 0.5mg | — | — | — | — | + | + | — | + | — |
| NOVOBIOCIN 0.5mg | — | — | — | + | + | + | — | + | — |
| CONTROL | — | — | — | — | — | — | — | — | — |

\+ induces malformations
— does not induce malformations

a decrease in rat and mouse fertility [12]. Chloramphenicol hemisuccinate given to stage 20 of development in doses of 2.5 mg and 5 mg/egg has a strong embryotoxic effect causing a death rate of 73% and 100% in the first half of the incubation period [4]. Rados [14] found anomalies



of the somites, endoderm, the nervous system and internal organs as a result of the action of chloramphenicol on the young embryo.

The mechanism of action of antibiotics on the embryonary development may involve perturbations in the process of proteic synthesis (Fig. 6). Penicillin inhibits the synthesis of the cellular wall and membrane and may lead to an increased frequency of telophases in the embryo [14]. Aminoglycosides, such as streptomycin, kanamycin, gentamycin, may inhibit the genetic message traduction. A result of their action is the incorrect insertion of the amino acids in the polypeptidic chain just being formed and thus aberrant proteins are synthetized. Chloramphenicol and Solvocillin bind to the 50 S subunits of the ribosome perturbing RNA-t activity. Novobiocin acts on the magnesium ions which are indispensable to the replicated DNA activity [15]. It is possible that by perturbing the multiple and highly intricated relationships between various protein synthesis stages, the fundamental processes implying cellular regulation be perturbed. As a result an insufficient amount of proteins is synthetized in extremely important periods of organogenesis, thus inducing malformations.

## CONCLUSIONS

The antibiotics tested by us have proved to have an embryotoxic and teratogenic effect on the chick embryo.

The following antibiotics were found to have an increased embryotoxicity: Solvocillin, Chloramphenicol, Novobiocin, Oxacillin ; they induced a mortality rate between 60 and 80%.

A teratogenic effect could be revealed in chicken treated with Chloramphenicol, Novobiocin, Solvocillin, Kanamycin, Oxacillin. The most severe malformations were exencephaly, meningocele, anophthalmy and eviscerations.

Our experimental study is a warning against the embryotoxic and teratogenic effects of some antibiotics in common use in humans.

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# THE ACTION OF DIFFERENT SYNTOFOLIN DOSES ON THE ACTIVITY OF SUPRAOPTICAL AND PARAVENTRICULAR HYPOTHALAMIC NUCLEI IN THE RAT

BY

ELENA MARCU

Two lots of white rats were hyperestrogenized by daily injections of 20 μg (lot A) and 100 μg (lot B) syntofolin, for 20 days.
Neurosecretory activity of the supraoptical and paraventricular nuclei was followed up in the course of the treatment. Syntofolin depressed neurosecretory activity function of the dose administered.

Removal of the gonads or the administration of sex hormones has been demonstrated to influence to a great extent the activity of magnocellular hypothalamic nuclei [4], [7], [13], [14], [15].

In an earlier work [11] we showed the changes that develop along the hypothalamo-neurohypophyseal (H—Nh) neurosecretory pathways following the continuous administration of syntofolin. Starting from the finding that summation with time of large syntofolin doses induces depression of neurosecretory activity in white rats of both sexes, we proposed to follow up the influence of syntofolin administered continuously and in different doses for a longer period of time.

## MATERIAL AND METHOD

Two lots (A, B), each of 15 white male rats, 150—170 g body weight, received a daily syntofolin dose of 20 μg (lot A) and 100 μg (lot B) by intraperitoneal route during 20 days. During administration of the hormone the rats were sacrificed at four days interval. The hypothalamic region of the brain was fixed in Halmi fluid and embedded in paraffin. The serial sections, 8 μ thick, were stained with paraldehyde-fuchsin in order to reveal the neurosecretion product [6]. The activity of the neurosecretory (NS) cells was estimated by measuring the magnocellular nuclei of 200 cells and calculating the mean nuclear diameter.

## RESULTS

*Reaction of the supraoptical (SON) and paraventricular nuclei (PVN) to 20 μg syntofolin (lot A).* In the control animals the NS cells are to be found in different phases of the secretory cycle and, according to the amount of neurosecretion present in the perikaryons in the SON (Fig. 1)

and the PVN (Fig. 7), two kinds of cells may be differentiated : (a) type I cells, large and medium with a voluminous nucleus, clear cytoplasm and few NS granules ; (b) type II cells, small, with a hyperchromatic cytoplasm due to the great number of NS granules. Type I cells are considered to be in an active state, and type II in a resting phase [5].

After *4 days* estrogenic treatment, the SON (Fig. 2) present a small amount of neurosecretion, most cells being of type I. In the PVN neurosecretion is somewhat greater (Fig. 8). A particular aspect is the great number of axons full of neurosecretion. Some, in the PVN exhibit a marked dilatation and are present both in the area of the hypothalamic nuclei and along the H—Nh pathways. This suggests the elimination of neurosecretion through the perikaryons, but also a deficient transport of this product along the axons.

At *8 days* (Fig. 4) neurosecretion is abundant in the axons and also increases in the perikaryons in both the SON and the PVN, in which case numerous type II cells can be observed.

At *12 days*, the aspect of the two nuclei differs : neurosecretion accumulates in the PVN and becomes more accentuated in the axons and perikaryons (Fig. 10), but decreases partially in the SON ; type II cells are rarer, and type I cells form small ergastoplasma plaques, which reflect the possibility of taking the secretory cycle up again.

At *16 days*, the SON perikaryons (Fig. 6) contain few NS granules ; most neurones are of type I, and the ergastoplasm becomes abundant at the periphery of the cells. This aspect is not maintained, however, because at *20 days* there is an evident reduction of ergastoplasm, a richer amount of neurosecretion in the cytoplasm and an increase in the number of full axons. In the PVN the situation differs : at *16 days* the PVN perikaryons are poor in ergastoplasm and the axons are full of neurosecretion ; at *20 days* the amount of NS granules decreases and the ergastoplasm zones become more evident (Fig. 11).

*Reaction of the SON and PVN to 100 µg syntofolin doses (lot B).* In contrast to lot A, after *4 days* treatment both the SON and the PVN revealed clear-cut aspects of neurosecretory accumulation in the perikaryons. In the SON, type II cells are predominant and the crinophores pathways more numerous than in the controls, but not to the same extent as in the lot A ; no dilatations were noted along the pathways. At *8 days* treatment the aspects were more or less similar in both nuclei, except for the full axons which were more numerous, and of variable size and aspect. In the PVN, especially in its terminal area, the axons exhibit dilatations full of compact NS granulations. Such axons are also frequently encountered along the H—Nh pathways (Fig. 12) and characterize (with slight variations) the entire period of estrogenic treatment.

→

Plate I. *Supraoptical nuclei (SON) aspect of the male white rats received daily 20 µg (dose A) and 100 µg (dose B) of syntofolin (oc. 10× : ob. 40× ).*

PLATE I

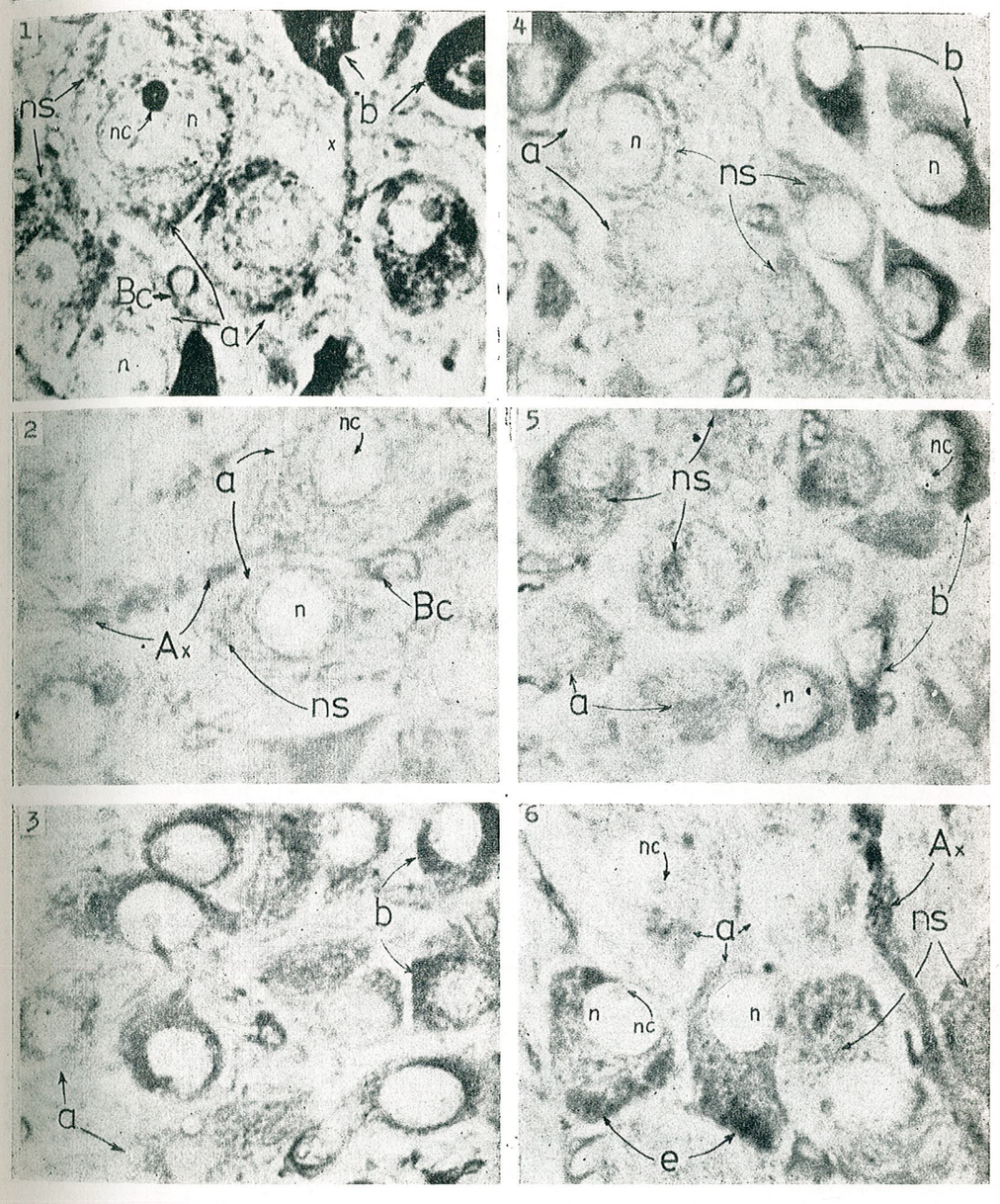
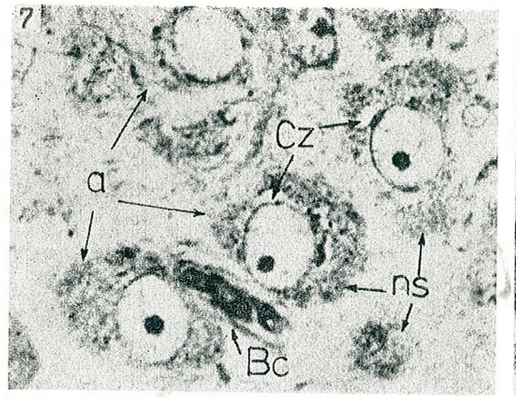
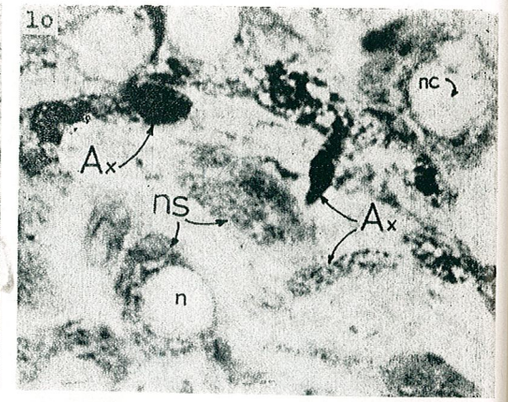
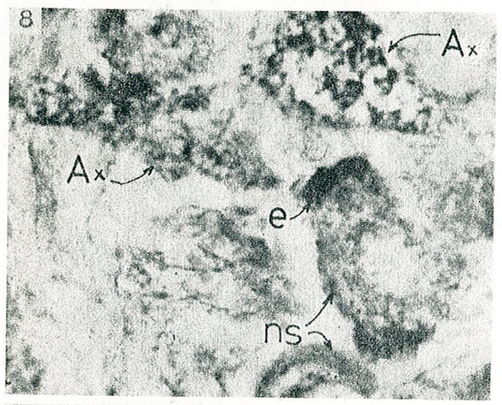
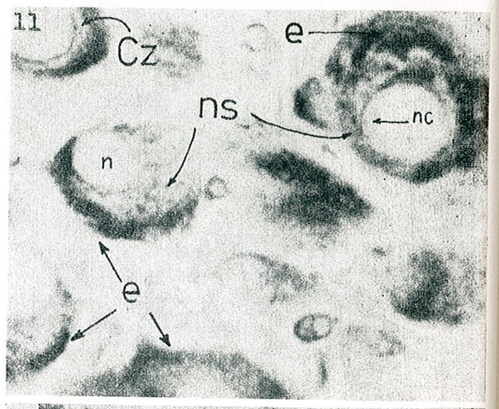


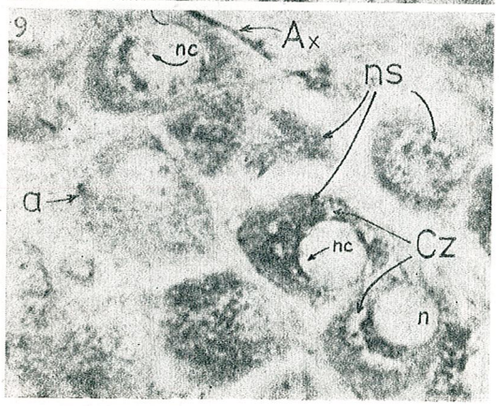

Fig. 1. — SON, in the control.
Fig. 2. — SON, at 4 days estrogenic treatment (dose A).
Fig. 3. — SON, at 4 days estrogenic treatment (dose B).
Fig. 4. — SON, at 8 days, estrogenic treatment (dose A).
Fig. 5. — SON, at 12 days estrogenic treatment (dose B).
Fig. 6. — SON, at 16 days estrogenic treatment (Dose A).
*a*, type I cells ; *b*, type II cells ; *n*, nucleus ; *nc*, nucleolus ; *e*, ergastoplasm ; *NS*, neurosecretory granules ; *AX*, axon fragments full of neurosecretion ; *Bc*, blood capillary.



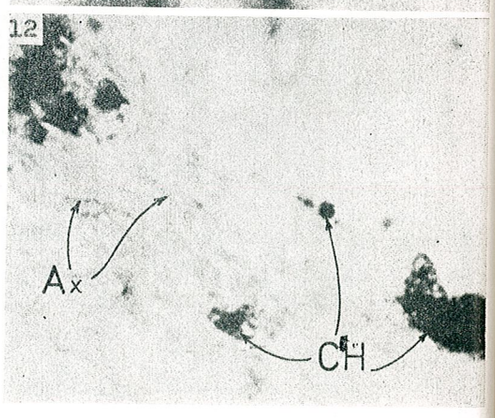




Fig. 7. — PVN, in the control.
Fig. 8. — PVN, at 4 days estrogenic treatment (dose A).
Fig. 9. — PVN, at 4 days estrogenic treatment (dose B).
Fig. 10. — PVN, at 12 days estrogenic treatment (dose A).
Fig. 11. — PVN, at 20 days estrogenic treatment (dose A).
Fig. 12. — hypothalamo-neurohypophyseal pathways at 8 days estrogenic treatment (dose B).
*a*, type cells; *b*, type II cells; *n*, nucleus; *nc*, nucleolus; *e*, ergastoplasm; *NS*, neurosecretory granules; *Cz*, clear zone empty of neurosecretory granules; *Ax*, axon fragments full of neurosecretion; *Bc*, blood capillary; *HC*, Herring body.



At *12 and 16 days* a significant decrease in neurosecretion takes place in both magnocellular nuclei (Fig. 5). The aspect and density of the full axons are maintained in the terminal zones of the SON and PVN wherefrom the H—Nh pathways arise. Neurosecretion discharge in the perikaryons continues up to *day 20* in the SON, whereas in the PVN it appears to have been arrested. The perikaryons with marked neurosecretion stain intensely.

*Table 1*

The average nuclear diameter of neurons from the hypothalamic SON and PVN nuclei in male white rats, daily treated with 20 μg and 100 μg syntofolin

| Administered dose | Hypothalamic nuclei | Average value % | Control | EXPERIMENT: Daily treatment with syntofolin for 20 days — Days of killing: IV | VIII | XII | XVI | XX |
|---|---|---|---|---|---|---|---|---|
| 20μg | SON | Average | 10.149 | 9.761 | 9.714 | 9.881 | 10.352 | 10.088 |
| | | % | | −3.824 | −4.287 | −2.641 | +2.000 | −0.602 |
| | PVN | Average | 9.750 | 9.402 | 9.043 | 8.901 | 9.763 | 9.852 |
| | | % | | −3.570 | −7.252 | −8.708 | +0.133 | +1.046 |
| 100μg | SON | Average | 10.149 | 9.053 | 9.520 | 9.413 | 9.840 | 9.991 |
| | | % | | −10.080 | −7.183 | −7.252 | −3.045 | −1.557 |
| | PVN | Average | 9.750 | 8.669 | 8.813 | 8.791 | 9.445 | 9.668 |
| | | % | | −11.088 | −9.611 | −9.836 | −3.129 | −0.831 |

*Caryometric data.* Nuclear measurements of the SON and PVN cells, in both lots of animals treated with syntofolin, showed that from the moment in which the treatment is applied and up to the 16th day, the mean nuclear diameter is below control values. This suggests that syntofolin reduces the NS biosynthesis potential, a phenomenon that affects to an equal extent the SON and PVN, but develops differently in terms of the dose administered, being more intense at a daily dose of 100 μg. On day 16 in lot A or day 20 in lot B, the mean nuclear diameter returns close to normal values (Table 1).

## DISCUSSIONS

The morphologic characters in the two magnocellular hypothalamic nuclei — the decrease in the size of NS cells, of the nuclei and nucleolus, the accumulation of neurosecretion in the perikaryons parallel to overloading of the nerve cell axons with this product, denote the reduction of NS activity. It is probable that a decrease in NS activity should

Plate II. *Paraventricular nuclei (PVN) aspects of the male white rats received daily 20 μg (dose A), and 100 μg (dose B) of syntofolin (oc. 10×; ob. 40×)*

be due to a state of maximum loading of the neurohypophysis with NS products, as in the case of mice hyperestrogenized for ten days [11].

The alterations are in general similar in both hypothalamic nuclei (SON, PVN), but the degree of depression of neurosecretion is directly proportional to the dose administered. The reactivity of PVN is more accentuated than in the SON, as also observed in mice [11]. PVN activity is more intensely depressed, which might denote a closer relationship with the "hypophysiotropic" area and the possibility of a closer correlation of these nuclei with the production of gonadotrophins [1], [3] [9]. The mechanism of these reactions may also imply the existence of certain neurons with receptors for sexual hormones situated in the anterior hypothalamic area and to a certain extent also in the PVN [2], [10], [12], [16].

Of interest in this experiment, in which the follow up time was doubled, is that starting on day 16 lot A showed a certain tendency to reestablish a neurosecretory activity in both nuclei studied. The neurons appear slightly hypertrophic, with a nucleus of relatively normal dimensions, with few NS granulations in the cytoplasm and organelles that mark the beginning of protein synthesis. Return to an activity close to the normal one probably denotes the tendency of these nuclei to adapt themselves to the hormonal stress, induced by the small hormone dose administered.

## CONCLUSIONS

1. Syntofolin reduces the biosynthesis activity of neurosecretory cells in both the supraoptical and the paraventricular hypothalamic nuclei.

2. Depression of the neurosecretion function takes place in correlation with and in proportion to the daily administered dose.

3. After an initial depression of neurosecretory activity, the supraoptical and paraventricular nuclei adapt themselves more or less to the hormonal stress and acquire a functional potential.

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# *IN VITRO* STUDY OF INTERACTIONS BETWEEN THE THYMUS AND THE TESTES USING TWO THYMIC EXTRACTS

BY

P. DESCHAUX, C. JOUVE DE GUIBERT and D. ARDAIL

Using two different thymic extracts, we have shown, *in vitro*, that thymus extracts controlled the growth of testes but had no effects on the secretion of Leyding cells.

The thymus participates in the defence of the organism by distributing into peripheral tissues so-called thymodependent T lymphocytes. These T cells are derived from hematopoietic cells which, under the influence of differentiation inducers secreted by the epithelial thymic cells, acquire certain immunological properties.

Using two different thymus principles, different in their biochemical composition and method of extraction, we showed in the rat that there was no action of the testes on the content of active principles of the thymus but on the weight. Furthermore, we showed an indirect role, *via* the pituitary, of the thymus on testosterone secretion and a direct role of the thymus on the weight of the testes [5] — [8]. In the work which we report here, we attempted to confirm *in vitro* the preceding obtained *in vivo*.

## MATERIAL AND METHODS

*Preparation of thymus extracts.* Two thymus extracts were prepared using calf thymus, according to two different techniques : Comsa et al's extract or HTH [2], [3]; and Goldstein et al's extract or thymosin fraction 5 [10]. These two extracts were proteins free of nucleic acid. The protein content was determined according to Lowry's technique [13].

*Cell cultures.* Cells isolated from a mashed organ or cultures, obtained with either trypsin solution (0.25%) or collagenase (0.05%) in Krebs solution, were maintained in medium 199 (Eurobio, France) to which 10% calf serum was added.

*Preparation of thymic epithelial cell cultures.* Male Sprague Dawley rats were sacrificed by decapitation at the age of 30 days. The thymus glands were removed aseptically, washed in Krebs solution containing antibiotics, and placed in a flask containing collagenase (0.05%) solution for one night at +4°C [9]. The collagenase was then eliminated by centrifugation and the thymus was cut into thin slices which were soaked in (0.25%) trypsin for 2 hours at +37°C. The suspension was

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then centrifuged at 700 × g for 10 minutes. The thymus fragments were distributed on the bottom of the culture flasks (Falcon 30 ml flasks). Medium 199 was added (5 ml). After 24 hours at +37°C, the medium was replaced by a larger quantity (20 ml). The thymus tissue adhered to the wall of the culture flask as a thin layer of epithelial cells. A supply of cells was harvested by carefully scrapping the bottom of the flask. The medium containing these cells in suspension was centrifuged (700 × g for 15 minutes). The pellet containing epithelial cells was used for DNA synthesis after verification of the structure of the cells under the electron microscope [9]. In order to identify the epithelial cell structure all preparations of thymus epithelial cell cultures were examined under the electron microscope before seeking the action of the testes. The characteristics of the thymus epithelial cells *in vivo* were found : desmosomes with tonofilaments and vacuoles which contain *material dense to electrons*. Furthermore, these cells possess an ergastoplasm and a dilated nucleus [9].

*Performance of Leydig cell cultures.* Sprague Dawley rats, 10 days old, were injected daily with human chorionic gonadotropin (Sigma) (100 µg ip in normal saline solution). These animals were killed by decapitation at the age of 20 days and the testes were removed aseptically. After decapsulation, the testes were transferred to a Rappaport type flask containing an isotonic saline solution plus antibiotics, and incubated with stirring at 37°C for 60 minutes. This gave separation of the seminiferous tubules from the intertubular fluid containing the Leydig cells. The latter were collected by filtration of the incubation medium using a transfusion system (type TR 116 M. Bruneau Laboratories). The filtrate was directly placed in culture flasks and incubated ; the medium was changed every 72 hours.

*The purity of these cells was determined by estimation of testosterone in the following way.* The nutrient medium used for the culture of Leydig cells was replaced by fresh medium containing 100 µg/ml of luteinising hormone. After 3 hours incubation at +37°C the medium was removed and the extracellular testosterone estimated by radioimmunoassay [8].

*DNA synthesis.* The cell suspension obtained previously was dispersed in 199 medium and distributed in glass hemolysis tubes at $10^6$ cells/ml/tube. The control tubes receive 0.1 ml of 199 medium, the reaction tubes 0.1 ml of medium containing in solution the substance used to study the testosterone effect, the 5 α-dihydrotestosterone (5 α DHT) and thymic extracts. This was allowed to incubate with continuous stirring in a water bath for 30 minutes at 37°C in a gas containing 5% $CO_2$ and 95% oxygen. Tritiated thymidine, 2 µ Ci/ml of the reaction medium, was added and the mixture was incubated 3 hours under the same conditions as the pre-incubation. The determination was the rate of incorporation of tritiated thymidine into cell DNA according to Munck's technique [14] adapted to cell suspensions. The results are expressed in cpm/$10^6$ cells.

*Action of the testosterone and 5 α DHT.* Three separate experiments were performed using 3 cell cultures with ten to twenty culture flasks. When a complete cell layer was complete, the cells are collected and distributed in hemolysis tubes : 5 control tubes, 10 tubes receiving the cell suspension + 0.1 mg of 5 α DHT. The DNA synthesis was then measured.



*Testosterone secretion by the Leydig cells.* The nurient medium covering the Leydig cell culture was replaced by a new culture medium containing the substance to be studied. After 3 hours incubation at 37°C, the medium was removed and the quantity of testosterone present determinated by radioimmunoassay [8]. The cells were then removed and counted.

## RESULTS

In 3 experiments (Fig. 1) testosterone significantly lowered the synthesis of DNA in the thymus epithelial cells. No effect was noted with 5 α DHT. Figure 2 shows the action of LH (100 ng/ml of medium) on the secretion of testosterone by the Leydig cells in culture. Note the increase in the secretion of testosterone without any increase in the number of cells.

The action of thymus extracts upon the secretion of testosterone by Leydig cells (Figure 3) changed according to the age of the cul-

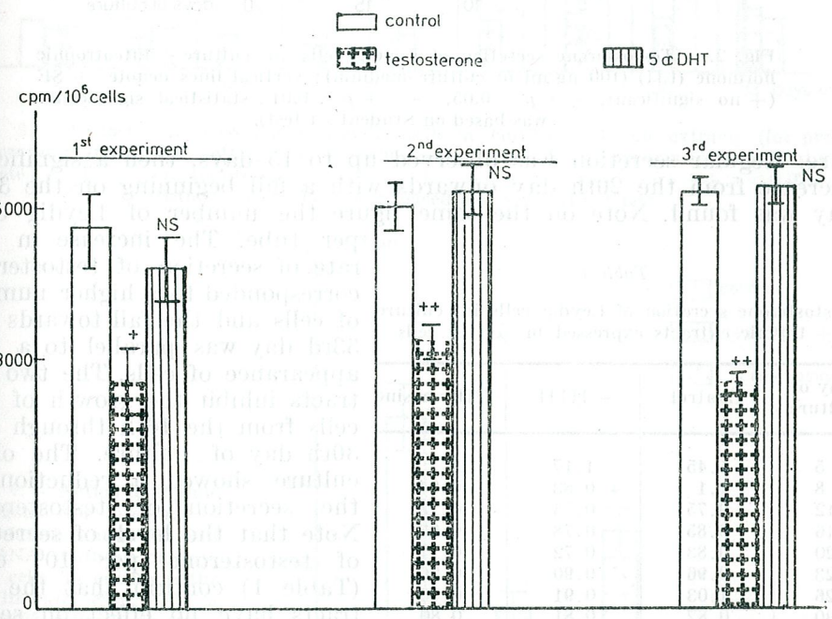


Fig. 1. — Action of testosterone and dihydrotestosterone (5 α DHT) on DNA synthesis of thymic epithelial cells ; results are expressed in cpm/$10^6$ cells ; the experiments were done 5 times ; vertical lines denote ± SE (+ $p < 0.01$, ++ $p < 0.001$, NS : no significance ; statistical significance was based on Student's t-test).

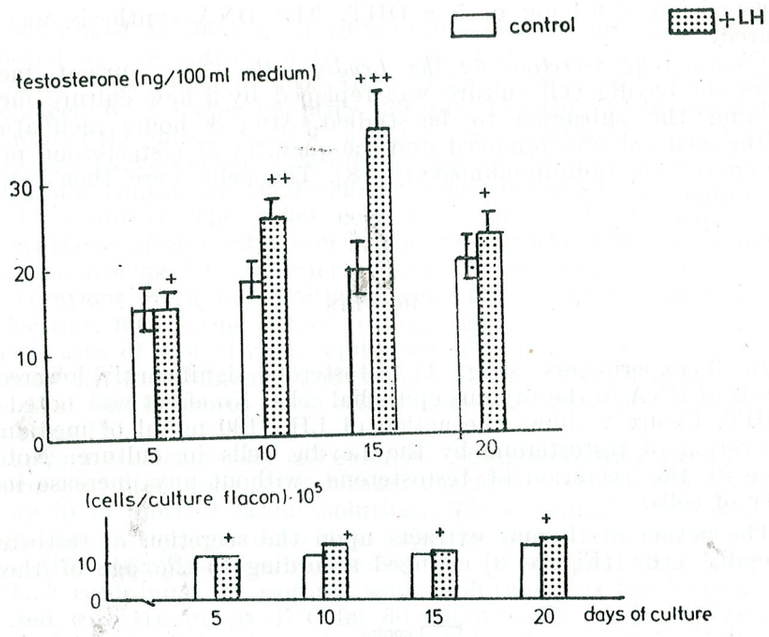


Fig. 2. — Testosterone secretion of Leydig cells in culture ± luteotrophic hormone (LH) (100 ng/ml of culture medium); vertical lines denote ± SE (+ no significant, ++ $p < 0.05$, +++ $p < 0.01$; statistical significance was based on Student's t test).

ture. Regular secretion was observed up to 15 days, then a significant increase from the 20th day onwards with a fall beginning on the 30th day was found. Note on the same figure the number of Leydig cells per tube. The increase in the rate of secretion of testosterone corresponded to a higher number of cells and the fall towards the 33rd day was parallel to a disappearance of cells. The two extracts inhibit the growth of the cells from the 16th through the 30th day of culture. The older culture showed a reduction in the secretion of testosterone. Note that the levels of secretion of testosterone per $10^6$ cells (Table 1) confirm that the extracts have no effect on secretion. The action of thymus extracts on DNA synthesis of the Leydig cells (Figure 4) indicated a definite inhibition of DNA synthesis in Leydig cells in the presence of thymus extracts.

*Table 1*

Testosterone secretion of Leydig cells in culture ± thymic extracts expressed in $\mu g/10^6$ cells

| Day of culture | Control | + HTH | + Thymosine |
|---|---|---|---|
| 5 | 1.45 | 1.17 | 1 |
| 8 | 1.1 | 0.83 | 0.94 |
| 12 | 0.75 | 0.75 | 0.78 |
| 16 | 0.85 | 0.78 | 0.93 |
| 20 | 0.83 | 0.72 | 0.77 |
| 23 | 0.96 | 0.90 | 0.96 |
| 26 | 1.03 | 0.91 | 0.96 |
| 30 | 0.87 | 0.81 | 0.80 |
| 33 | 0.75 | 1.23 | 0.94 |



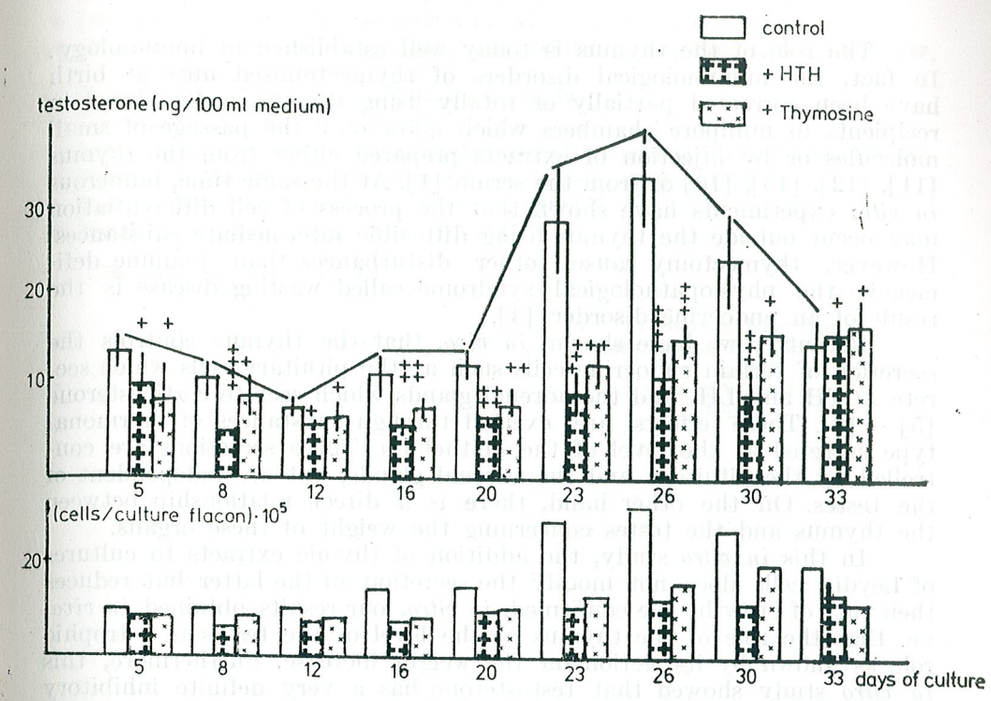


Fig. 3. — Testosterone secretion of Leydig cells in culture ± thymic extracts (for preparation see Material and methods). Each value is the mean of five experiments, vertical lines denote ± SE. (+ no significant, ++ $p < 0.1$, +++ $p < 0.05$, ++++ $p < 0.02$, statistical significance was based on Student's t-test).

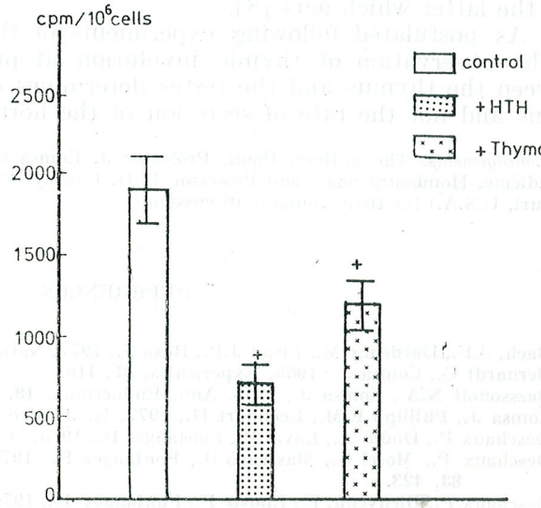


Fig. 4. — Action of thymic extracts (for preparation see Materials and methods) on DNA synthesis of Leydig cells in culture; the results are expressed in cpm/$10^6$ cells. Each value is the mean of five experiments; vertical lines denote ± SE. (+ $p < 0.01$; statistical significance was based on Student's t test).

## DISCUSSION

The role of the thymus is today well established in immunology. In fact, the immunological disorders of thymectomised mice at birth have been corrected partially or totally using thymus grafts placed in recipients in millipore chambers which allow only the passage of small molecules or by injection of extracts prepared either from the thymus [11], [12], [15], [16] or from the serum [1]. At the same time, numerous *in vitro* experiments have shown that the process of cell differentiation may occur outside the thymus using diffusible intermediate substances. However, thymectomy causes other disturbances than immune deficiency, the physiopathological syndrome called wasting disease is the result of an endocrine disorder [4].

Recently, we have shown, *in vivo*, that the thymus controls the secretion of certain endocrine cells such as the pituitary cells which secrete ACTH and LH, and the adrenal glands which secrete corticosterone [5] — [8]. These effects are exerted through substances of hormonal type secreted at the level of the epithelium. These secretions are controlled by the pituitary and the adrenal glands, but are independent of the testes. On the other hand, there is a direct relationship between the thymus and the testes concerning the weight of these organs.

In this *in vitro* study, the addition of thymic extracts to cultures of Leydig cells does not modify the secretion of the latter but reduces their rate of growth. We confirmed, *in vitro*, our results obtained *in vivo*, i.e. that the role of the thymus at the level of the testes is a trophic role as shown by its action on the weight increase. Furthermore, this *in vitro* study showed that testosterone has a very definite inhibitory effect on the synthesis of DNA of the thymus epithelial cells. No effect was found with 5 α DHT. *In vivo*, these two steroids exert an equivalent inhibitory effect on the weight of the thymus. One may consider that the 5 α DHT injected *in vitro* is transformed into testosterone and that it is the latter which acts [8].

As postulated following experiments of the removal of glands or by the observation of thymic involution at puberty, the interrelation between the thymus and the testes determines only the weight of these organs and not the rate of secretion of the hormones.

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# RADIOPROTECTIVE EFFECTS OF MADIOL AND LEUCOTROFINA STUDIED BY THE DYNAMIC CHANGES OF SOME METABOLIC PROCESSES IN THE THYMUS AND THE LIVER OF X-IRRADIATED RATS

BY

MARIA BORŞA, A.D. ABRAHAM and Z. URAY

Male Wistar rats were treated with Madiol, irradiated with a single dose of 400 R(X) and injected p.r. with Leucotrofina (active polypeptides isolated from the calf thymus). Dynamic changes of thymus and liver glycogen, lipid and protein bios ynthesis showed remarkable radioprotective effects of these substances, which stimulate the recovery processes in whole-body X-irradiated rats.

Anabolic steroids enhance resistance to total body X-irradiation and have moderate radioprotective effects [3], [6]. Some thymus extracts, such as thymosin and leucotrofina (a cell-free calf thymus extract containing active polypeptides), which were able to stimulate lymphocytopoiesis and to increase the immunological competence, were also found to be effective radioprotective agents [9], [11], [12], [13], [14].

The aim of this study was to investigate the effect of treatment with Madiol and Leucotrofina on the metabolism of the thymus and the liver by whole body X-irradiated rats. In an earlier work [6] we reported that some changes in nucleic acid and protein metabolism induced by X-irradiation of mice whole body could be prevented by a.r. administration of Madiol.

## MATERIAL AND METHODS

Male Wistar rats weighing 130—160 g were fed with standard food at room temperature. The animals were exposed to whole body X-irradiation with a single dose of 400 R and killed on the 3th, 8th and 15th day after exposure. Other group of animals received a.r. 30 mg Madiol (Biofarm, Bucharest) per 100 g body weight *(per os)* for a period of 30 days.

After irradiation the animals were treated with 1 ml Leucotrofina (Ellem-Spa, Milano) per 100 g body weight. Leucotrofina was injected i.m. after 1 hr and every second day until 3, 8, 15 days.

Glycogen content was determined with Montgomery's technique [7]. Protein concentration was determined with Lowry's method [5]. Some animals received 1 hr before killing 2 $\mu$Ci of (2-$^{14}$C) acetate (sodium salt) and the rate of incorporation of radiocarbon into proteins and lipids was determined after extraction of these substances from tissues with the aid of Folch's technique [4], dissolving the proteins in 30 per cent

KOH and in Bray's solution and the lipids in T-fluor solution. The specific radioactivity of these substances was determined at 10°C using a liquid scintillation spectrometer (BF—5003). The results were evaluated by Chauvenet's and Student's statistical methods.

## RESULTS

The results obtained showed that thymus glycogen content increased significantly after 3 and 15 days of exposure (Table 1). Liver glycogen

*Table 1*

Changes of glycogen content in the thymus and the liver of X-irradiated and treated rats. (Concentration of glycogen is expressed in mg per 1 g fresh tissue)

| | Control | X-irradiated group | | | M-X-L-group | | |
|---|---|---|---|---|---|---|---|
| | Days | 3 | 8 | 15 | 3 | 8 | 15 |
| X | 0.96 | 1.26 | 1.06 | 1.60 | 1.14 | 1.25 | 1.07 |
| ±SE | 0.05 | 0.11 | 0.08 | 0.14 | 0.07 | 0.12 | 0.06 |
| D% | — | +31.25 | +10.42 | +66.67 | +18.75 | +30.21 | +11.46 |
| P | — | <0.01 | >0.25 | <0.001 | >0.10 | >0.5 | >0.25 |
| X | 12.85 | 39.49 | 30.78 | 69.26 | 25.58 | 24.75 | 31.04 |
| ±SE | 1.35 | 3.45 | 4.96 | 9.88 | 6.04 | 2.76 | 1.60 |
| D% | — | +207.32 | +139.53 | +438.99 | +99.07 | +92.61 | +141.56 |
| P | — | 0.001 | 0.01 | 0.001 | 0.05 | 0.001 | 0.001 |

content increase after exposure was more accentuated in comparison with the thymus glycogen under the influence of irradiation, suggesting a strong stress effect of the ionizing radiation.

The treatment with Madiol and Leucotrofina caused no change of glycogen content in the thymus and only a moderate increase in the liver after irradiation in comparison with the non-irradiated control (Table 1).

The rate of (2-$^{14}$C) acetate incorporation into the thymus lipids showed the same dynamic change as observed in the case of thymus glycogen: after 3 days the rate of incorporation of radiocarbon into lipids increased with 198.57 per cent, after 8 days the value was appropriated to the control value, and after 15 days an increase of 176.81 per cent was observed (Fig. 1). It is important to note that the rate of biosynthesis of lipids in the liver increased only after 8 days of exposure with 130.32 per cent (Fig. 1). Lipid biosynthesis in the thymus of treated animals did not change in comparison with the control, but the rate of incorporation of radiocarbon into lipids in the liver enhanced about five times after 8 days. After 15 days no significant difference of lipid biosynthesis in the liver in comparison with the control (fig. 1) was noticed.

Proteins concentration and their biosynthesis in the thymus and the liver of irradiated and treated animals were studied by the determination of the total protein concentration and of the rate of conver-



sion of (2-$^{14}$C) acetate into proteins synthesized *de novo*. The results obtained showed that X-irradiation caused a significant decrease of protein concentration in the thymus of irradiated animals. In the thymus after 3 days of exposure a significant decrease (—22.91 percent) was observed, and the concentration of proteins remained under the control

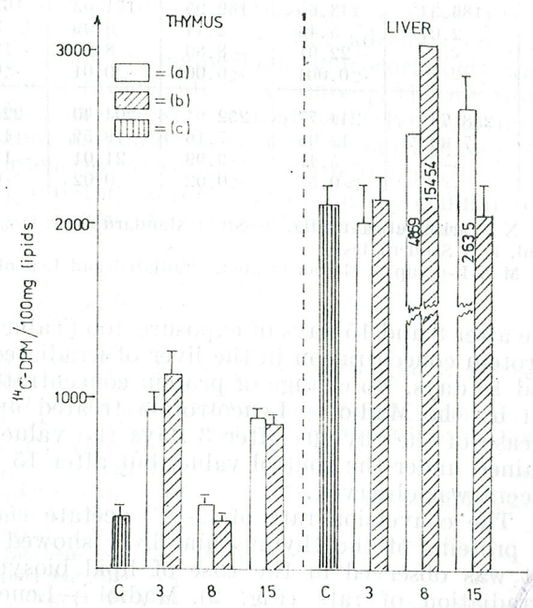


Fig. 1. — Dynamic changes of the incorporation rate of (2-$^{14}$C) acetate into lipids of the thymus and the liver of rats after X-irradiation (a) and treatment with Madiol + Leucotrofina (b) in comparison with the control (c).

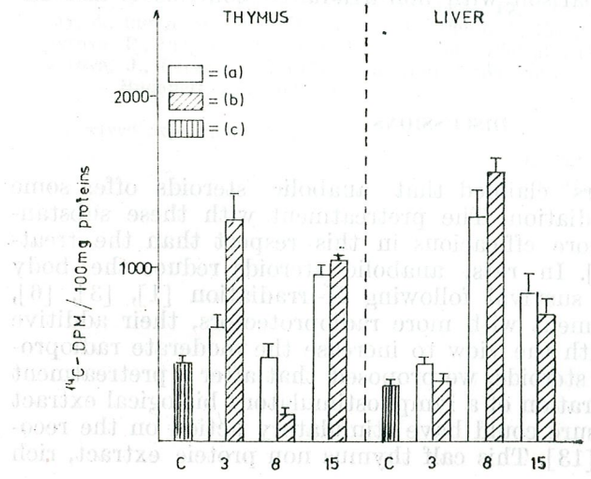


Fig. 2. — Dynamic changes of the conversion rate of (2-$^{14}$C) acetate into proteins of the thymus and the liver of rats after X-irradiation (a) and treatment with Madiol + Leucotrofina (b) in comparison with the control (c).

*Table 2*

Changes of protein concentration in the thymus and the liver of X-irradiated and treated rats. (Protein concentration is expressed in mg per 1 g fresh tissue)

| | Control | X-irradiated group | | | M-X-L-group | | |
|---|---|---|---|---|---|---|---|
| | Days | 3 | 8 | 15 | 3 | 8 | 15 |
| X | 186.34 | 143.65 | 169.95 | 171.03 | 162.35 | 186.23 | 226.45 |
| ±SE | 2.01 | 5.46 | 2.44 | 5.09 | 8.89 | 11.38 | 11.66 |
| D% | – | −22.91 | −8.80 | −8.22 | −12.87 | −0.06 | +21.53 |
| P | – | <0.001 | <0.001 | <0.01 | <0.02 | >0.5 | <0.001 |
| X | 258.78 | 244.77 | 232.91 | 204.40 | 228.91 | 241.68 | 259.53 |
| ±SE | 7.96 | 12.08 | 7.16 | 19.59 | 13.21 | 13.67 | 15.96 |
| D% | – | −5.41 | −9.99 | −21.01 | −11.54 | −6.61 | +0.29 |
| P | – | >0.5 | <0.02 | <0.02 | >0.5 | >0.25 | >0.5 |

X = arithmetical media, ± SE = standard error, D% = per cent difference as against control, P = Student test

M-X-L-group = Madiol treated, irradiated and Leucotrofina treated rats.

value after 8 and 15 days of exposure too (Table 2). A significant decrease of protein concentration in the liver of irradiated rats was observed after 8 and 15 days. No change of protein concentration in the liver was registered by the Madiol + Leucotrofina treated and irradiated animals. In the case of the thymus after 3 days the value of protein concentration remained under the control value, but after 15 days an increase of 21.53 per cent was observed.

The conversion rate of (2-$^{14}$C) acetate *via* $^{14}$C-labelled amino acids into proteins of the thymus and liver showed similar dynamic changes as it was observed in the case of lipid biosynthesis, after whole body X-irradiation of rats (Fig. 2). Madiol + Leucotrofina caused stimulation of protein biosynthesis especially on the 3th day of exposure and a decrease on the 8th day. 15 days after exposure the stimulation of protein biosynthesis in comparison with non-irradiated controls is also noticed.

## DISCUSSIONS

Several investigators claimed that anabolic steroids offer some protection against X-irradiation. The pretreatment with these substances is allegedly much more efficacious in this respect than the treatment after exposure [2]. In rats, anabolic steroids reduce the body weight loss and increase survival following X-irradiation [1], [3], [6], [10]. Following the treatment with more radioprotectors, their additive effects were observed. With the view to increase the moderate radioprotective effects of anabolic steroids, we proposed that after a pretreatment with Madiol, the administration of a lymphostimulatory biological extract (Leucotrofina) after exposure could have stimulatory action on the recovery systems [11], [12], [13]. This calf thymus non proteic extract, rich



in active polypeptides, stimulates the maturation of bone marrow cells and the increase of leukocytes under circulation [15].

Our results showed an evident stimulation of the recovery processes in the thymus and the liver of X-irradiated animals, and demonstrated clearly that Madiol + Leucotrofina administered a.r., respectively p.r., prevented the changes of glycogenoneogenetic pathways and the protein and lipid biosynthesis induced by irradiation.

Some authors elaborated hypotheses concerning the action of anabolic steroids which may be mediated through the adrenals [10] and inhibition of the catabolic action of endogenous glucocorticoids which are secreted in elevated concentrations after exposure [10]. Our results showed that administration of an anabolic steroid and a lymphostimulatory factor to irradiated animals could prevent some biochemical alterations induced by radiation energy at the level of the lymphatic system and of the liver, by their stimulatory action exerted on the cellular recovery systems and by the inhibition of catabolic steroids action.

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# DYNAMIC CHANGE OF NUCLEIC ACIDS IN THE THYMUS AND THE LIVER OF X-IRRADIATED RATS AFTER TREATMENT WITH NON-TOXIC RADIOPROTECTORS

BY

ŞTEFANIA MANCIULEA, MARIA BORŞA, A.D. ABRAHAM and CAMELIA BANU

A single dose of 400 R(X) determined a marked decrease of DNA and RNA concentration in the thymus and the liver of whole-body irradiated rats. The treatment with Madiol + Leucotrofina prevented the degradation of nucleic acids and stimulated the recovery processes in both organs.

In an earlier work [3] we reported that Madiol has a moderate radio-protective effect. Uray et al [7] showed that Leucotrofina exerts excellent hemopoietic and lymphostimulatory effects.

The aim of this study was to investigate the effect of an anabolic steroid (Madiol) and of a lymphostimulatory factor (Leucotrofina) on X-irradiated rats. The dynamic change of nucleic acids in the thymus and the liver was studied after irradiation and treatment.

## MATERIAL AND METHODS

Male Wistar rats weighing 130—160 g were kept in standard conditions. The experimental animals were divided in the following groups: — control non-irradiated group (C); X-irradiated groups — animals killed after 3 days ($X_3$), 8 days ($X_8$), and 15 days ($X_{15}$) of exposure; X-irradiated groups treated a.r. *per os* with 30 mg Madiol (Biofarm, Bucharest) per 100 g body weight during 30 days (2 mg Madiol on every second day). These animals were treated also p.r. with 1ml. Leucotrofina (Ellem-Spa, Milano) per 100 g body weight, injected i.m. 1 hr after exposure and killed on the 3th day ("$MXL_3$"). Other group was injected with Leucotrofina at 1, 48, 72 and 120 hr and killed on the 8th day ("$MXL_8$"). Another group was injected on every second day with Leucotrofina till the 13th day and killed on the 15th day after exposure ("$MXL_{15}$").

A single dose of 400 R(X) was applied with the aid of a therapeutic unit TUR X (180 kV, 10 mA, 1 mm Cu. FSD 80 cm, 30 R/min). The dose rate was measured with the aid of a Siemens universal dosimeter.

DNA and RNA concentrations were determined in the thymus and the liver using Spirin's differential spectrophotometrical method [5] and a Zeiss V.S.U.-1 type apparatus.

## RESULTS AND DISCUSSION

The absolute and relative weight of the thymus on the 3th day after exposure is diminished, all glands presenting involution aspects, observed by other authors too [1]. On the 8th or 15th day the weight of the thymus remained under control value, but higher than on the 3th day.

The treatment with Madiol + Leucotrofina did not influence this change of glands in comparison with the animals irradiated.

The DNA concentration in the thymus after irradiation showed characteristic dynamic changes. After 3 days of exposure an obvious decrease of DNA concentration was observed (Table 1), which is due to

*Table 1*

Dynamic change of DNA and RNA concentration in the thymus of irradiated (X) and treated rats (MXL). (Results are expressed in μg/mg fresh tissue)

| | | C | $X_3$ | $X_8$ | $X_{15}$ | $MXL_3$ | $MXL_8$ | $MXL_{15}$ |
|---|---|---|---|---|---|---|---|---|
| DNA | $\bar{X}$ | 20.13 | 6.34 | 16.99 | 19.22 | 6.03 | 19.66 | 20.09 |
| | SE | ±0.42 | ±0.34 | ±1.80 | ±0.31 | ±0.61 | ±0.86 | ±0.62 |
| | n | 7 | 8 | 8 | 7 | 8 | 7 | 6 |
| | % | | −68.50 | −15.60 | −4.52 | −69.80 | −2.33 | −0.20 |
| | p | | <0.001 | >0.1 | >0.05 | <0.001 | >0.1 | >0.1 |
| RNA | $\bar{X}$ | 8.19 | 6.60 | 5.68 | 10.58 | 3.73 | 6.64 | 8.57 |
| | SE | ±0.24 | ±0.38 | ±0.29 | ±0.31 | ±0.55 | ±0.46 | ±0.27 |
| | n | 8 | 8 | 7 | 8 | 8 | 8 | 7 |
| | % | | −19.41 | −30.65 | +29.18 | −54.46 | −18.93 | +4.64 |
| | p | | <0.001 | <0.001 | <0.001 | <0.01 | <0.05 | >0.05 |

the immediate stress effect of ionizing radiation. After 8 or 15 days DNA concentration remained under the control value (C group), but the differences are only slightly significant. The administration of Madiol + Leucotrofina caused no essential change of DNA concentration in the thymus of the irradiated group as against the nonirradiated one (Table 1). The results concerning the dynamic change of RNA concentration

*Table 2*

Dynamic change of DNA and RNA concentration in the liver of irradiated (X) and treated rats (MXL). (Results are expressed in μg/mg fresh tissue)

| | | C | $X_3$ | $X_8$ | $X_{15}$ | $MXL_3$ | $MXL_8$ | $MXL_{15}$ |
|---|---|---|---|---|---|---|---|---|
| DNA | $\bar{X}$ | 4.35 | 2.71 | 4.61 | 3.91 | 4.50 | 4.45 | 3.88 |
| | SE | ±0.12 | ±0.24 | ±0.24 | ±0.17 | ±0.16 | ±0.17 | ±0.09 |
| | *n* | 8 | 8 | 7 | 8 | 8 | 8 | 8 |
| | % | | −37.70 | +5.98 | −10.11 | +3.45 | +2.30 | −10.80 |
| | *p* | | <0.001 | >0.25 | <0.05 | >0.1 | >0.1 | <0.01 |
| RNA | $\bar{X}$ | 6.41 | 5.73 | 4.42 | 5.98 | 5.11 | 4.77 | 5.64 |
| | SE | ±0.12 | ±0.23 | ±0.23 | ±0.28 | ±0.11 | ±0.27 | ±0.12 |
| | *n* | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | % | | −10.61 | −31.05 | −6.71 | −20.28 | −25.59 | −12.01 |
| | *p* | | <0.01 | <0.001 | >0.05 | <0.001 | <0.001 | <0.001 |

in the thymus demonstrate a more rapid return to the normal values, in the case of irradiated and treated animals, than in irradiated and non-treated rats (Table 1).

The DNA concentration of the liver after irradiation decreased significantly only after 3 days of exposure. On the 8th day the same



order of diminution of RNA concentration was observed (Table 2). No essential change of DNA concentration on the 3th and 8th day was observed with "$MXL_3$" and "$MXL_8$" groups. However, a significant decrease of RNA concentration was registered by these groups. On the 15th day only moderate changes were observed in all irradiated groups in comparison with the non-irradiated controls.

The change in nucleic acids (DNA and RNA) in the thymus and the liver is in strong relation with the physiological status of these organs [6]. The immediate reactivity of the thymus to the stress effect of ionizing radiations could be explained by the stimulation of the capacity of adenohypophysis of secreting ACTH [2], [4]. ACTH exerts a stimulatory effect on the glucocorticoid hormones synthesis and secretion, which determines thymus involution after irradiation. The treatment with Madiol + Leucotrofina prevents a dramatic change of DNA and stimulates the recovery processes in the thymus. Our results prove that this treatment exerts a protective effect on the DNA in the liver, but does not prevent the loss of RNA in the first 8 days after exposure, which suggests that the RNA-synthetic apparatus of hepatocytes remained affected.

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# CONTRIBUTION TO THE STUDY OF RADIOBIOLOGICAL EFFECTS OF NEUTRONS

BY

C. VLĂDESCU, M. CÂRSTEANU, S. GROSU, I. DOROBANȚU

In this paper the authors have studied the influence of whole body irradiation of rats, with different doses of neutrons in serum proteins and plasmatic free amino acids, by ion-exchange and thin layer chromatography. The irradiation of animals leads to modifications in the concentration of serum proteins and of free amino acids, in relation with the dose size used.

The effects of neutrons on the living body is a question of great interest for all peoples. The studies presented in 1978 in Jülich (FRG) at The Fourteenth Annual Meeting of European Society of Radiation Biology have pointed out the importance of these investigations [15].

The investigations in neutron radiobiology proved that the neutron irradiation of organism leads to a slight cellular repair, and the protectors control only to a small extent the restoration process [8], [10]. For this purpose it is necessary to find some biological limits of irradiation before the appearance of effects which are detectable by usual methods. In our laboratory the effect of whole body irradiation was investigated with neutrons on serum proteins and free amino acids from the plasma of experimental animals.

## MATERIAL AND METHODS

Animals utilized : Wistar rats, 10—12 months old and 150 g weight. The animals were irradiated with 600, 300, 200, and 150 rad fast neutrons and sacrificed after 1, 2, 3, 4, 5 and 30 days. Irradiation source : beryllium target bombarded with deuterons accelerated to cyclotron for an energy of 13.5 MeV. The neutron flow, corresponding to a current of 1 $\mu$A accelerated deuterons, was $\Phi = 2 \cdot 10^8$ n/cm$^2$·s. The dosimetric equivalent of the neutron flow was 1 rad/s and corresponded to a flow of $2 \cdot 10^8$ n/cm$^2$·s. The dose flow rate was 0.81 rad/s.

In the conditions in which the deuteron current intensity was the same, the variation of the dose will be given by the variation of the exposure time (in our case 6 minutes for a dose of 600 rad, 3 minutes for a dose of 300 rad, 2 minutes for a dose of 200 rad and 1.5 minutes for a dose of 150 rad neutrons).

The ion-exchange chromatography method of Morris and Morris [11] was used for the separation of serum proteins, on a DEAE-Sephadex A-50 column. The proteins are eluted with increasing concentrations of natrium chloride (0.07 M, 0.17 M, 0.37 M) in phosphate buffer, pH 6.6.

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Samples of 5 ml were collected with an LKB automatic collector and the protein concentration was determined by measuring the specific absorbance at 280 nm.

The plasmatic free amino acids were determined by thin layer chromatography [12], [14] and by ion-exchange chromatography in an aminoanalyser of the type Chinoin 1975.

Glass-plates of 20 × 20 cm are covered with a Kieselgel G layer of 0.2 mm. Samples of 4 µl are two-dimensionally chromatographed utilizing as migration solvents butanol-acetic acid-water in a volumetric proportion of 4 : 4 : 1 and respectively n-butanol — methyl cetone — water in a volumetric proportion of 5 : 3 : 1. The development solution was 0.3% ninhydrine in n-butanol diluted with the solvent number 1 in proportion of 1 : 8. The identification of amino acids is achieved by measuring the Rfs and comparing the colour intensity of spots. For ion-exchange chromatography Dowex 50 X is used. Samples of 1 ml supernatant of hydrolyzed plasma are added in an aminoanalyser (the plasma is hydrolyzed with TCA in a volumetric proportion of 2 : 1). The solvents used are citrate buffer pH 3.28, 4.28 and 6.0.

The identification of amino acids is achieved spectrophotometrically at 570 nm for the majority of amino acids and 440 nm for proline.

## RESULTS

The serum proteins separated from normal rats (control) by ion-exchange chromatography on DEAE-Sephadex, in our work conditions, exhibit three fractions denoted by A, B and C (Fig. 1).

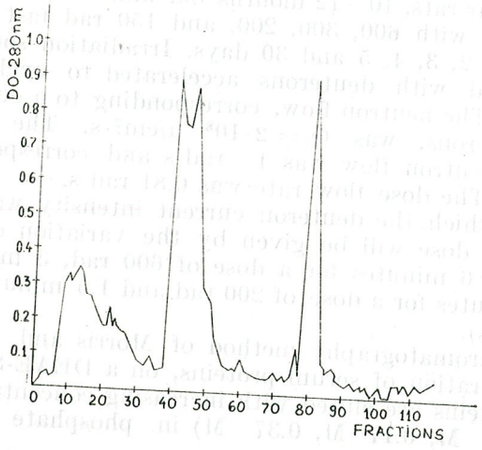


Fig. 1. — Chromatogram of serum proteins in normal rats (control)

The protein concentration of fraction A in normal rat serum has an optical density at 280 nm between 0.3 and 0.4 and includes 150 ml



| DOSE | TIME AFTER IRRADIATION | | | | | |
|---|---|---|---|---|---|---|
| | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours | 30 days |
| 600 rad | B/C < 1 | B/C > 1 | B/C ≃ 1 | — | — | — |
| 300 rad | B/C > 1 | B/C > 1 | B/C > 1 | B/C < 1 | — | — |
| 200 rad | — | B/C > 1 | B/C ⩽ 1 | — | B/C ⩽ 1 | — |
| 150 rad | B/C ≃ 1 | B/C ≃ 1 | B/C ≃ 1 | B/C ≃ 1 | — | B/C ≃ 1 |
| CONTROL | B/C = 1 | | | | | |

Fig. 2. — The ratio between serum protein peaks B and C in irradiated rats.

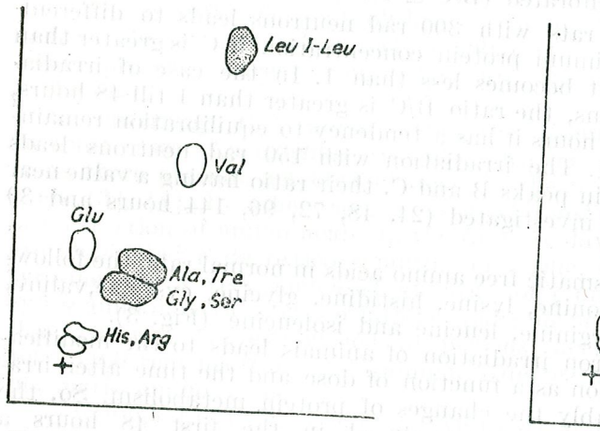


Fig. 3. — Thin layer chromatography of plasmatic free amino acids in normal rats (control).

Fig. 4. — Thin layer chromatography of plasmatic free amino acids in rats irradiated with 200 rad neutrons 48 hours after irradiation.

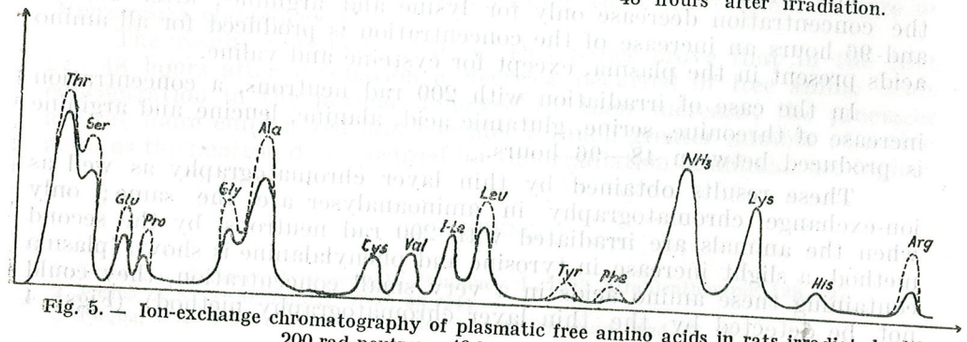


Fig. 5. — Ion-exchange chromatography of plasmatic free amino acids in rats irradiated with 200 rad neutrons, 48 hours after irradiation.

eluent with a peak between 45 and 48 ml. The second fraction B, corresponding to serum proteins extracted with 0.17 M sodium chloride, includes 65 ml and exhibits a peak between 200 and 235 ml with an optical density of 0.9. The third fraction, namely fraction C, includes 75 ml and has a peak between 390 and 415 ml corresponding to an optical density of 0.95.

The irradiation of animals with fast neutrons leads to changes of serum protein concentration in all three fractions, especially in fractions B and C, at all doses in this experiment. Therefore, it is to be noticed the modification of the concentration ratio between protein fractions B and C under the influence of neutrons.

The column chromatography on DEAE-Sephadex of normal blood serum leads to the two fractions B and C with approximately equal peaks ($B/C \cong 1$) (Fig. 2). 24 hours after neutron irradiation of rats with 600 rad, the ratio of the two peaks is less than one ($B/C < 1$); after 48 hours, the ratio becomes greater than one ($B/C > 1$) and after 72 hours it is again equilibrated ($B/C \cong 1$).

The irradiation of rats with 300 rad neutrons leads to different results: the ratio of maximum protein concentration B/C is greater than 1 until 96 hours when it becomes less than 1. In the case of irradiation with 200 rad neutrons, the ratio B/C is greater than 1 till 48 hours, so that after 72 and 120 hours it has a tendency to equilibration remaining slightly less than 1. The irradiation with 150 rad neutrons leads to little changes of protein peaks B and C, their ratio having a value near unity every time when investigated (24, 48, 72, 96, 144 hours and 30 days).

Concerning the plasmatic free amino acids in normal rats, the following were revealed: threonine, lysine, histidine, glycine, cysteine,valine, serine, glutamic acid, arginine, leucine and isoleucine (Fig. 3).

The effect of neutron irradiation of animals leads to the modification of their concentration as a function of dose and the time after irradiation, following probably the changes of protein metabolism. So, the irradiation with 600 rad neutrons produced in the first 48 hours a decrease of concentration for all the amino acids mentioned, followed after 72 hours by an increase of their concentration (except for cysteine).

The effect of irradiation with 300 rad neutrons after 2 hours is the concentration decrease only for lysine and arginine; after 48, 72, and 96 hours an increase of the concentration is produced for all amino acids present in the plasma, except for cysteine and valine.

In the case of irradiation with 200 rad neutrons, a concentration increase of threonine, serine, glutamic acid, alanine, leucine and arginine is produced between 48—96 hours.

These results obtained by thin layer chromatography as well as ion-exchange chromatography in aminoanalyser are the same; only when the animals are irradiated with 200 rad neutrons, by the second method, a slight increase in tyrosine and phenylalanine is shown (plasma containing these amino acids in a very small concentration, they could not be detected by the thin layer chromatography method) (Figs. 4 and 5).



The irradiation of rats with 150 rad leads to a slow increase of concentration only after 72 hours in the case of serine, glutamic acid, leucine and arginine. The chromatograms obtained 30 days after irradiation had in all cases the same pattern as in the control.

## DISCUSSION

Serum proteins represent from the metabolic point of view a mirror of the changes which occur in the organism. The investigations referring to the X-ray irradiation of experimental animals, whole-body irradiated, pointed out a significant increase in total serum proteins and especially of α-and β-globulin fractions [13]. Henneberg [6] established a decrease of albumin fraction. The literature is scarce about the neutron action on serum protein. However, Donev and co-workers [1] established an increase of seric β-lipoproteins in chronically irradiated persons. Evans [2] mentioned modifications of the concentration in seric glycoproteins in dogs irradiated with 250—450 rad neutrons and gamma radiations.

The modifications established by us in this paper prove that the neutron action on whole-body irradiation is reflected in changes of serum proteins concentration, which are the more evident the greater is the dose.

Concerning the amino acids, the literature data are sporadic. In some cases of accidental irradiation with gamma radiations and neutrons there are not detected changes in the urinary excretion of amino acids [3], [4]. In other cases it is established that by urinary cumulate excretion of amino acids in the first six days after irradiation, there are eliminated a decreased quantity of serine, threonine, ethanolamine, tyrosine and a very increased quantity of glutamic acid, aspartic acid and γ-aminobutyric acids, ornithine and taurine [9]. It is worth noticing that Latarjet [7] established in persons, accidentaly irradiated with 400—1100 rem neutrons and gamma, a decrease of the concentration in plasmatic amino acids.

Grivina [5] pointed out in investigations concerning the influence of gamma radiations and neutrons on amino acid biosynthesis on *Micrococcus glutamicus* culture, that the mutants produced under the radiation action, synthetize 2—3 times more lysine, 2—5 times more glutamic acid and about 4 times more alanine than the original culture of *Micrococcus glutamicus*.

The results obtained by us in this paper prove that in the first 24—48 hours after irradiation a decrease occurred of free amino acids concentration in the plasma, followed by their increase; the processes are the more emphasized and consider a much greater number of amino acids as the neutron doses utilized for the irradiation of animals are higher.

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# DDT EFFECTS ON THE MEMBRANE PHASE TRANSITIONS INDUCED BY CHANGES IN EXTERNAL $Ca^{2+}$ CONCENTRATION

BY

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Experiments have been performed on frog sartorius muscle fibres by the intracellular glass microelectrodes technique, where the effects of DDT ($5.10^{-4}$M) on the phase transitions induced by increased external $Ca^{2+}$ concentrations (membrane hyperpolarization) as well as by a $Ca^{2+}$-free medium (membrane depolarization) have been observed. The insecticide prevents $Ca^{2+}$ binding in the globular phospholipidic micella and facilitates $Ca^{2+}$ removal from the laminar phospholipidic micella. In both cases DDT preferentialy interacts with the micella less sensitive with respect to the transitional action of $Ca^{2+}$.

It is a long time since the influence of $Ca^{2+}$ on the DDT-induced repetitive afterdischarges in crustacean nerves has been indicated [8], [19]. An increase of the $Ca^{2+}$ concentration in the external medium, generally suppresses the DDT-induced repetitive afterdischarges. This observation is taken as indicating that DDT disturbs the binding of $Ca^{2+}$ with the membrane components, thereby causing an unstabilizing effect.

More recently, based on the results obtained by changes in the external $K^+$ concentration [3], [5], it was suggested that the DDT effects on the membrane might have as an explanation the ability of the insecticide to prevent the $Ca^{2+}$ action on the phospholipidic micella in the external membrane layer. For obtaining new evidence in favour of this idea, in this work the DDT effects on membrane potential in the conditions of $Ca^{2+}$ concentration increase in the medium, as well as in a $Ca^{2+}$-free medium, have been observed.

## MATERIAL AND METHODS

The membrane potential of the frog *(Rana ridibunda)* sartorius muscle single fibres was determined by the intracellular glass microelectrodes technique, using a "Tonnies" oscilloscope for amplification and recording.

The high-$Ca^{2+}$ Ringer solution ($Ca^{2+}$ 12 times more than in the normal Ringer) was prepared by equimolar substitution of $Na^+$ for $Ca^{2+}$. The $Ca^{2+}$-free Ringer was prepared by equimolar substitution of $Ca^{2+}$ for $Na^+$.

The DDT concentration used was $5 \cdot 10^{-4}$M and was obtained by dissolving the insecticide in ethanol first, and then adding it to the Ringer, so that the final ethanol concentration did not exceed 1%.

## RESULTS

In the high-$Ca^{2+}$ Ringer (without DDT) a membrane hyperpolarization was noticed, with a rapid onset in the first 2 minutes, then con-

tinued with a lower speed and reaching a maximum amplitude of 10 mV after 30 minutes (Fig. 1 — Control).

At the reestabishment of the normal $Ca^{2+}$ concentration, a decrease of the membrane potential in the first 5 minutes was observed, but the membrane still remained hyperpolarized with about 5 mV for a long time.

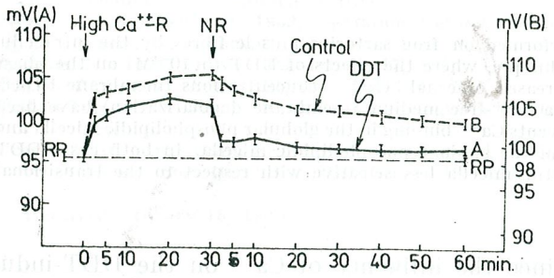


Fig. 1. — DDT effect on membrane hyperpolarization in high-$Ca^{2+}$ medium.

In the presence of DDT the high-$Ca^{2+}$ induced hyperpolarization was also rapid, but was clearly lower in amplitude (5.7 mV after 30 minutes) (Fig. 1—DDT).

Reestablishment of the normal $Ca^{2+}$ concentration led to an initial tendency of repolarization, which was even more pronounced than in the absence of DDT; however, the normal resting potential was not reached at within an hour.

The $Ca^{2+}$-free Ringer induced a membrane depolarization of 7.1 mV after 30 minutes (Fig. 2-Control). The onset of the phenomenon was slower than in the case of high-$Ca^{2+}$-induced hyperpolarization. When the membrane was reintroduced in normal Ringer the repolarization was very rapid and complete in 10 minutes.

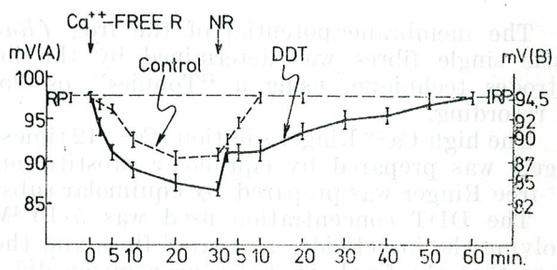


Fig. 2. — DDT effect on membrane depolarization in $Ca^{2+}$-free medium.

The DDT presence in $Ca^{2+}$-free Ringer determined an increase in the speed and amplitude of the membrane depolarization (Fig. 2-DDT).

The membrane repolarization in normal Ringer after such a depolarization was slowed down by the insecticide (except for the first 2 minutes), so that the initial resting potential was reached at just after an hour.



## DISCUSSIONS AND CONCLUSIONS

There are numerous works where it was pointed out that the removal of $Ca^{2+}$ from the external medium led to a depolarization [6], [7], [10], [17], and the increase of $Ca^{2+}$ concentration led to a hyperpolarization of the membrane [11], [12], [14]. In the latest ones, the effects of external calcium concentration variations havealready been interpreted as resulting in fact from the modification of the $K^+ : Ca^{2+}$ ratio.

But the explanations concerning the mechanism of the antagonism between these two cations at the membrane level have been less satisfactory. It has been admitted that the variation of the $K^+ : Ca^{2+}$ ratio in the external medium determines a molecular conformational modification of the membrane proteins and has a series of correlated effects with respect to the membrane electrical potential and permeability [20]. Other authors, having in mind the ion exchanger properties of the membrane phospholipids and their capacity to modify their supermolecular organization by phase transitions [13], [18], considered these membrane components to be the actual support of the interactions between $K^+$ and $Ca^{2+}$.

On the basis of a membrane model previously described [1], [2], numerous explanations concerning the mechanisms of these phenomena have been elaborated and, implicitly, the interactions between DDT and external $Ca^{2+}$, described in this work, have been clarified.

Considering that the membrane phospholipids play an essential part in the bioelectrical and in the permeability phenomena, the idea that both the depolarizations and the hyperpolarizations of the membrane induced by the external $K^+ : Ca^{2+}$ ratio modification are based on the phase transitions of the phospholipidic micella from the external membrane layer was advanced.

Membrane depolarization by the external $Ca^{2+}$ concentration diminution (increase of the $K^+ : Ca^{2+}$ ratio) has been explained by the phase transition of the cationic laminar micella to the anionic globular micella, with the loss of $Ca^{2+}$ from the structure and the binding of $K^+$ to it [1], [15].

The mechanism of membrane hyperpolarization by external $Ca^{2+}$ concentration increase (decrease of the $K^+ : Ca^{2+}$ ratio) is a phase transition of anionic globular micella to cationic laminar micella, with the loss of $K^+$ from the structure and the binding of $Ca^{2+}$ to it [1], [16].

The DDT effects on these phenomena, induced by the external $K^+ : Ca^{2+}$ ratio modification, either by an increase or by a decrease of $Ca^{2+}$ concentration, presented in this paper, confirm our previous idea [3], [5] that the insecticide is able to prevent the $Ca^{2+}$-binding to globular micella, and to suppress in this way their phase transition to laminar micella.

This clearly emerged both from the evolution of the high-$Ca^{2+}$ induced hyperpolarization (Fig. 1) and from the development of the recovery after $Ca^{2+}$free Ringer induced depolarization (Fig. 2).

However, in both cases, the onset of the phenomena is not considerably affected (it remains rapid enough in the presence of DDT), and only their subsequent development is slowed down. This proves that DDT does not prevent equally the $Ca^{2+}$-binding and $Ca^{2+}$ re-binding to

all globular micella which undergo the phase transition to laminar micellae.

In order to bring further explanations of several membrane phenomenon aspects, it has been previously admitted [1], [4] a differentiation of phospholipidic micellae from the point of view of their sensitivity to the destructuring ion action. These are all the more numerous as they bind more weakly the structuring ion and are more easily affected by the destructuring ion (by lower concentrations of the latter). And there are all the less numerous as they most strongly bind the structuring ion and are more hardly affected by the destructuring ion (by higher concentrations of the latter).

The most sensitive globular micellae to $Ca^{2+}$ concentration increase (and thus the most numerous ones), and the first to undergo the phase transition, are not so much considerably influenced by DDT, as are the ones less sensitive to the transitional action of $Ca^{2+}$.

The idea of phospholipidic micellae differentiation with respect to their degree of sensitivity to DDT might be correlated with the observation made by Hille [9] that the DDT-treated membrane behaves as some of their $Na^{+}$-channels, open and close normally, whereas others close with much delay after they were opened.

Although the author deals with this problem on the basis of another membrane model, his observation points to the fact that DDT does not affect to the same extent all the membrane parts responsible for the permeability passive changes.

The experiments performed also indicated that DDT has the effect of promoting the $Ca^{2+}$-loss from the laminar micellae as well. But in this case the sensitivity of laminar micellae toward DDT is differentiated. This is presented by the evolution of the $Ca^{2+}$-free medium induced depolarization (Fig. 2) and of the recovery after the high-$Ca^{+2}$ induced hyperpolarization (Fig. 1), when the beginning of the phenomena is accelerated by DDT.

Or, it is just these phenomena that begin with the phase transition of the micellae less sensitive to $Ca^{2+}$ and less numerous, which have a higher sensitivity to the insecticide.

The results obtained by the modification of external $K^{+}$ concentration [3], [5] also support the *idea that DDT interacts preferentialy with membrane phospholipidic micella more resistive to the transitional* $Ca^{2+}$ *action.*

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# GRISEOFULVIN ACTION ON MEMBRANE POTENTIAL IN NORMAL MEDIUM AND IN DIFFERENT $Ca^{2+}$ CONCENTRATIONS

BY

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Investigations were performed on frog sartorius muscle fibres in normal Ringer and in $Ca^{2+}$-free Ringer or with 5 mM $Ca^{2+}$(pH=7.2). The effects of griseofulvin (0.25 mM) are correlated with its lipophilic properties. Membrane depolarization under normal ionic conditions can be explained by the interference of the antibiotic with active transport. The effects upon phase transitions of the phospholipids are differentiated. When the $K^+ : Ca^{2+}$ ratio is diminished griseofulvin reduces the transitional efficiency of $Ca^{2+}$. When this ratio is increased $Ca^{2+}$ binding to structure increases as well.

In many papers dealing with the action of antibiotics, their specific effect has been shown as being achieved either at the level of some intracellular metabolic processes, or at the level of cellular membranes. But even in the case of some intracellular action there also take place some effects on the properties of membrane or on the transport of ions and other substances through it [6], [9], [13]. More interaction ways between antibiotics and some direct or indirect membrane phenomena were thus emphasized [3], [9], [10].

In the case of griseofulvin, where the very mechanism of the total action has not been yet established [7], [14], the way of the intimate action at the level of the cellular membrane has been known even less.

Taking this fact into account we have followed in this paper the influence of griseofulvin on the membrane potential in normal external medium and with a modified $K^+ : Ca^{2+}$ ratio (by the change of $Ca^{2+}$ concentration), with the aim to clear out its interference with the membrane components implied in the bioelectrical phenomena.

## MATERIAL AND METHODS

The experiments were performed on the frog sartorius muscle fibres *(Rana ridibunda*, Pall.), by the method of the glass intracellular microelectrodes. Each experiment was conducted on five muscles at room temperature. We used a Ringer solution with bicarbonate buffer (pH = 7.2). The $Ca^{2+}$-free solution was obtained by the equimolar replacement of this ion by $Na^+$, and the solution with $Ca^{2+}$ (5 mM) was prepared by the substitution of an equimolar quantity of NaCl for $CaCl_2$.

The griseofulvin solution (0.25 mM) was prepared by adding the substance to a Ringer solution containing 1% dimethylylformamide (DMFA) for the solubilization of the antibiotic. The statistical significance was calculated by Student's test.

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## RESULTS

The resting potential under normal conditions had an average value ranged between 92.64 mV and 93.50 mV (ES about 0.50 mV value) (Figs. 1—3 : RP).

In the experiments in which we followed the 0.25 mM griseofulvin effect on the membrane potential in normal Ringer with 1% DMFA (pH = 7.2), we registered a depolarization of the muscle fibre membrane with a rapid onset and an average amplitude of 4.86 mV (Fig. 1). This depolarization is maintained throughout the whole period of treatment (60 minutes) and is reversible on washing the fibres with normal Ringer (NR) in about 30 minutes.

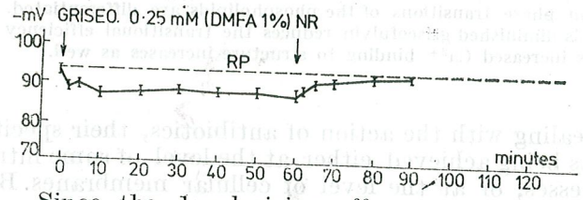


Fig. 1. — Griseofulvin effect on the membrane potential, in normal Ringer.

Since the depolarizing effect sets on rapidly (about 2 minutes), it has to be considered as a result of the direct action of the antibiotic upon the membrane and not as indirectly deriving from some action at the intracellular level.

We used a small quantity of griseofulvin (0.25 mM) since at a higher concentration (1 mM) a stronger and irreversible depolarization is obtained.

The organic solvent (DMFA) has no obvious effect upon the membrane potential with the concentration used by us (1%). Later griseofulvin effects upon the membrane potential were followed in the conditions of changing the $K^+ : Ca^{2+}$ ratio by the modification of calcium concentration.

In the experiments in which fibres were initially maintained in $Ca^{2+}$-free Ringer there can be noticed a depolarization of the membrane with an average amplitude of 9.59 mV (Fig. 2—A), phenomenon described by us in a previous paper [11]. On introducing the fibres into Ringer with 0.25 mM griseofulvin a marked delay of the repolarization is found out, in comparison with the experiments without antibiotic [11]. The resting value of the potential is reached after only 60 minutes.

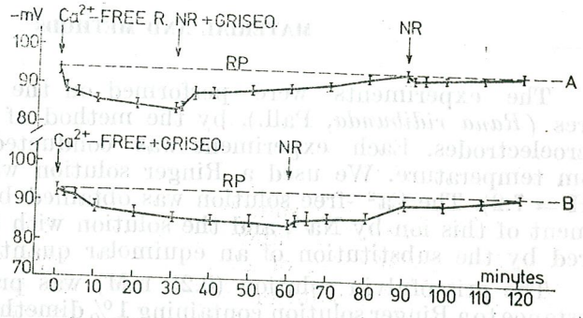


Fig. 2. — Griseofulvin action on the membrane potential in $Ca^{2+}$-free Ringer.



The depolarization of the muscle fibre membrane in $Ca^{2+}$-free Ringer is also influenced by griseofulvin (0.25 mM), the speed and the amplitude of the phenomenon being much reduced (Fig. 2—B) in comparison with the depolarization when the antibiotic is absent (Fig. 2—A). By replacing the fibres back into normal Ringer, the persistence of the action of the antibiotic bound to the membrane is to be found out, the repolarization being much delayed, like in the experiment presented above.

In high-$Ca^{2+}$ Ringer, as we showed in another paper as well [12], a hyperpolarization of the membrane with an average amplitude of 6.60 mV is found (Fig. 3—A). On washing the fibres with Ringer containing griseofulvin (0.25 mM), the membrane potential does not come back to the normal value, as in the experiments without antibiotic [12]. The hyperpolarization is maintained almost unchanged for an hour, recovery being of only 1.11 mV. Not even the later washing with Ringer without antibiotic can lead to a faster recovery of the normal potential.

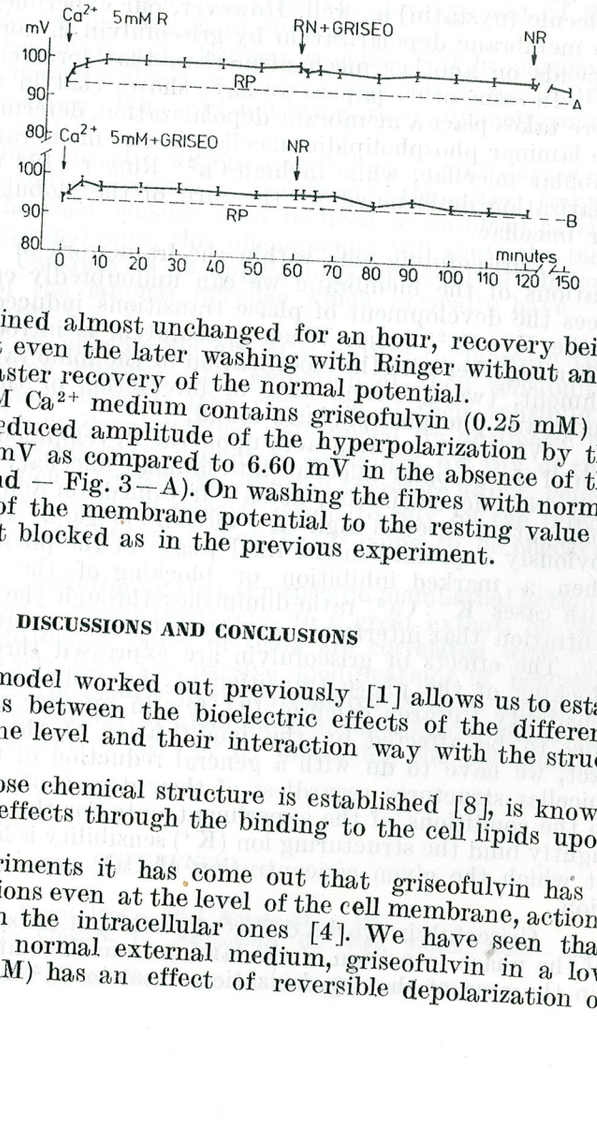


Fig. 3. — Griseofulvin action on the membrane potential in 5 mM $Ca^{2+}$ Ringer.

When the 5 mM $Ca^{2+}$ medium contains griseofulvin (0.25 mM) as well (Fig. 3—B), a reduced amplitude of the hyperpolarization by the increased $Ca^{2+}$ (3.44 mV as compared to 6.60 mV in the absence of the antibiotic is to be found — Fig. 3—A). On washing the fibres with normal Ringer the recovery of the membrane potential to the resting value is much delayed but not blocked as in the previous experiment.

## DISCUSSIONS AND CONCLUSIONS

The membrane model worked out previously [1] allows us to establish some correlations between the bioelectric effects of the different agents at the membrane level and their interaction way with the structure of the latter.

Griseofulvin, whose chemical structure is established [8], is known to achieve its specific effects through the binding to the cell lipids upon which it a cts [14].

From our experiments it has come out that griseofulvin has a series of important actions even at the level of the cell membrane, actions that are distinct from the intracellular ones [4]. We have seen that in the conditions of a normal external medium, griseofulvin in a low concentration (0.25 mM) has an effect of reversible depolarization of

the striated muscle fibre membrane. Since the depolarization effects of the membrane, induced by different external agents, rely especially on their interaction with the phospholipids in the membrane [11], we can also admit that the depolarizing effect of griseofulvin derives from its molecule strong lipophilia [8]. Our observation that griseofulvin used in higher concentration (1 mM) has an effect of irreversible lithic depolarization of the membrane suggests its tendency of accumulation in the latters' hydrophobic phase, finally leading to the structure disorganization.

Besides, there have been found out by us and other authors [5] similar effects upon the membrane charge (reversible depolarization in low concentrations and lithic depolarization in high concentrations) in the case of some other antibiotics with a stressed lipophilia of the molecule (nystatin) as well. However, our experiments have proved that the membrane depolarization by griseofulvin in normal external medium depends on another mechanism than that foreseen for nystatin.

In some other papers we have shown that in $Ca^{2+}$-free Ringer [11] there takes place a membrane depolarization, determined by the change of the laminar phospholipidic micellae in the membrane external layer into globular micellae, while in high-$Ca^{2+}$ Ringer [12] there occurs a hyperpolarization determined by the turn of the globular micellae into laminar micellae.

If griseofulvin acts within the framework of such structural modifications of the membrane we can undoubtedly establish that it influences the development of phase transitions induced by the modification of the $K^+ : Ca^{2+}$ ratio. When griseofulvin is introduced to the medium either together with the modification of the ionic ratio, or on its re-establishment, two different ways of interaction of the antibiotic with the membrane phospholipids are found.

When griseofulvin acts upon the development of the phase transition of the globular micellae into laminar micellae, as in the case of recovery from depolarization in $Ca^{2+}$-free medium and in the case of hyperpolarization onset in high-$Ca^{2+}$ medium (Figs. 2—A and 3—B) an action obviously expressed in the final phase of the phenomenon is found out, when a marked inhibition or blocking of the latter takes place. In both cases $K^+ : Ca^{2+}$ ratio diminishes through the increase of $Ca^{2+}$ concentration that interferes as a destructuring agent of the globular micellae. The effects of griseofulvin are expressed through the inhibition or blocking of the phase transition of the globular micellae with reduced sensibility towards calcium [2], few in number and which are the last ones to be affected by the modification of the ionic ratio. While, in fact, we have to do with a general reduction of the sensibility of these micellar structures regardless of their degree of sensibility at $Ca^{2+}$. But in the conditions of the experiment only for the globular micellae which tightly bind the structuring ion ($K^+$) sensibility is lowered under the value at which the given concentration of $Ca^{2+}$ can induce the phase transition.

Griseofulvin works differently when it acts upon the development of the phase transition of the laminar micellae into the globular micellae (in the case of the depolarization onset in $Ca^{2+}$-free medium and in the



case of the recovery from hyperpolarization induced by high external $Ca^{2+}$) (Figs. 2—B and 3—A).

The clearly expressed action of the antibiotic is obvious this time in the final phase of the phenomenon as well and it is also expressed through its marked inhibition or blocking.

However, experiments take place in the conditions of increasing the $K^+ : Ca^{2+}$ ratio, the alkaline ion acting as a destructuring agent of laminar micellae. Griseofulvin would not apparently influence the phase transition of the micellae with a reduced sensibility towards $K^+$, but it would stop the transitional action of $K^+$ upon the micellae with a higher sensibility for it. The phenomenon can be, however, explained if we admit that griseofulvin has an effect of general rise of binding $Ca^{2+}$ to all the laminar micellae of whatever degree of sensibility. In these conditions, when the $K^+ : Ca^{2+}$ ratio increases, the development of the phenomenon will be affected by two peculiarities :

— in the phase transition only laminar micellae will be engaged, i.e. those which are more sensible towards $K^+$ and whose sensibility, even diminished due to griseofulvin, will not bring about an inefficiency of the real concentration of $K^+$.

— as these micellae are also differentiated in point of degree of sensibility and number on the degree of sensibility, the phase transition setting on from the least sensible (and reduced in number) to the most sensible (and numerous) ones, this phenomenon will show the tendency of a slow onset. Contrary to all appearances griseofulvin hinders the phase transition of the laminar micellae which normally have a reduced sensibility towards $K^+$.

Since the actions of griseofulvin are of a nature to increase the stability of both globular micellae (the reduction of their sensibility towards $Ca^{2+}$) and laminar micellae (the reduction of their sensibility towards $K^+$), the membrane depolarization determined by antibiotic in conditions of a normal resting state cannot be explained by any of the agent's effects upon the phase transitions. We can therefore admit, generally speaking, the determination of depolarization to rely on the interference with the active ion transport, a fact that is going to be checked up experimentally.

We can conclude by underlining that the specific membranal actions of griseofulvin are complex. All of them rely, to a great extent, on the high lipophilia of the antibiotic. Some of them are correlated with the active transport and others with the passive modifications of permeability and electric charge. The passive modifications result from the interference with the phase transitions of membrane phospholipids, either by reducing the transition effect of $Ca^{2+}$ or by increasing the ion binding degree in the structure.

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# PROCAINE EFFECT ON MEMBRANE DEPOLARIZATION IN HIGH-POTASSIUM MEDIUM

BY

I. NEACȘU and ȘT. AGRIGOROAEI

Experiments were performed on frog sartorius muscle fibres in Ringer containing 30 mM $K^+$ and 2.5 mM procaine. at pH = 7.2 and pH = 6. In alkaline medium procaine causes a delay of $K^+$ mediated depolarization, whereas in acid medium both a a delay as well as a decrease of depolarization amplitude. The repolarization in normal Ringer is very slow, especially at pH = 7.2, due to the persistence of procaine within the membrane. Cationic procaine exhibits its effects due to competition with membrane $Ca^{2+}$. The uncharged form stabilizes the globular micellae.

In papers concerning the correlation between potassium and procaine action on the cell membrane it has been shown that procaine may reduce the speed and amplitude of the depolarization, caused by external high-potassium [20], [21] and may lead to a only partial restauration of the depolarization, if added to the medium after the establishment of the potassium effect [6], [23]. It has also been shown that the depolarization by external high potassium may be inhibited by an increase of external calcium concentration [14], [22] and that procaine may simulate the role of $Ca^{2+}$ at the membrane level [9], [18]. The author's conclusions seem however, contradictory, not suggesting a clear correlation between these observations.

In order to elucidate these aspects we followed up the time course of the membrane depolarization by external high-$K^+$ in the presence of procaine and of the corresponding repolarization in normal Ringer, at pH = 7.2 and pH = 6, respectively.

## MATERIAL AND METHODS

Experiments were performed on the membrane of *(Rana ridibunda*, Pall.) sartorius muscle fibres by the glass microelectrodes technique. Each experiment was conducted on five muscles at room temperature. Normal Ringer solution was prepared with bicarbonate buffer for pH = 7.2 and with phosphate buffer for pH = 6. The solution with 30 mM $K^+$ and 2.5 mM procaine was obtained by substituting in the Ringer solution an equimolar amount of NaCl for KCl and by addition of the anesthetic. The statistical significance was calculated using Student's test.

## RESULTS

In the experiments performed at pH = 7.2, the mean value of the intial resting potential was 91.96 mV (ES $\pm$ 0.25 mV) (Fig. 1 : RP). In Ringer containing 30 mM $K^+$ and 2.5 mM procaine a membrane depo-

larization is observed (Fig. 1—A) which takes place much slower than the depolarization in the absence of the anesthetic [19], especially during the first phase. Thus, after 5 minutes the depolarization amplitude (22.44 mV) is 14.16 mV lower than in the absence of procaine, the average speed of depolarization being also more reduced (4.48 mV/min as compared to 7.32 mV/min). The depolarization amplitude is, after 15 minutes (31.88 mV), 8.72 mV lower than in the absence of the anesthetic and

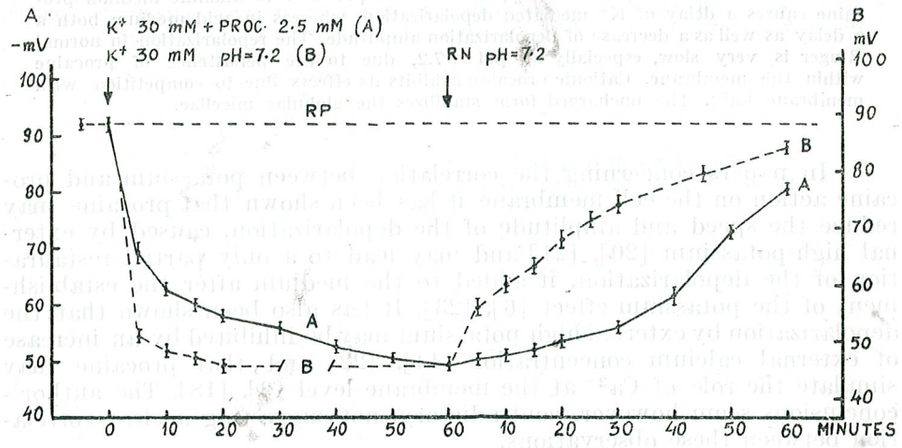


Fig. 1. — Membrane depolarization in 30 mM $K^+$ and 2.5 mM procaine Ringer and repolarization in normal Ringer, at pH = 7.2.

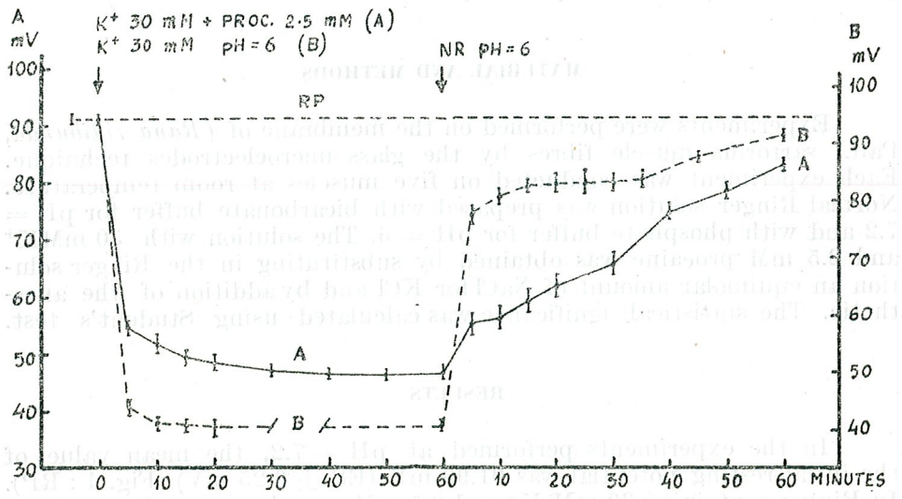


Fig. 2. — Membrane depolarization in 30 mM $K^+$ and 2.5 mM procaine Ringer and repolarization in normal Ringer, at pH = 6.



becomes approximately equal to this one only after 60 minutes. Bringing back the fibres into normal Ringer with normal $K^+$ and without procaine, a much slower membrane repolarization is found as compared to the control experiments [19], especially during the first 30 minutes. As a matter of fact, in a previous paper [6] we showed that a repolarization inhibition is also observed when procaine is added to normal repolarization Ringer, if the membrane was already depolarized by $K^+$.

The experiments performed at pH = 6 were conducted on fibres with a normal resting potential of 91.42 mV (ES ± 0.21 mV) (Fig. 2 : RP). Although at this pH the $K^+$ mediated membrane depolarization is more intense [19], the presence of procaine (2.5 mM) in the 30 mM $K^+$-Ringer solution causes a strong inhibition of the membrane depolarization (Fig. 2—A).

After 5 minutes the depolarization amplitude (37.14 mV) is 13.36 mV lower than the one obtained in the absence of procaine (Fig. 2—B) [19], the average depolarization speed being decreased too (7.43 mV/minute as compared to 10.10 mV/minute). The amplitude of the phenomenon after 15 minutes (42.30 mV) is 11.60 mV lower as compared to the experiments without procaine and stays approximately at the same value for 60 minutes. When the fibres are washed with normal Ringer the membrane repolarization is extremely slow as compared to the depolarization experiments in the absence of procaine, but quicker during the first phase than at pH = 7.2.

## DISCUSSIONS AND CONCLUSIONS

It is known that a change of the $K^+ : Ca^{2+}$ ratio in favour of $K^+$ leads to a membrane depolarization [4], [13], [18], [22]. In previous papers [18], [19] we showed that this depolarization is based on a phase transition of the laminar phospholipidic micellae from the external membrane layer into globular micellae, due to the replacement of $Ca^{2+}$ by $K^+$ in the structure. The amplitude of the phenomenon is influenced by the external pH. We also showed that procaine (2.5 mM) in Ringer with a normal $K^+ : Ca^{2+}$ ratio produces a membrane hyperpolarization [7].

Our experiments indicate that procaine applied simultaneously with external high-$K^+$ influences the speed and amplitude of the membrane depolarization. This procaine induced effect is similar to the $Ca^{2+}$ effects, since the $K^+$ depolarization may be slowed down or reduced by raising the external $Ca^{2+}$ concentration [12], [14], [16], [22]. It has been pointed out that addition of procaine to a high-$K^+$ medium is equivalent to an increase in the $Ca^{2+}$ concentration [10], [11]. Our previous results [7], [18] also showed that procaine has the capacity to partially simulate the role of calcium.

The interpretation of our results was based on the "2—M.S.I." concept, regarding the membrane structure and properties [2], [3], and

the complex interaction between procaine and the membrane structure was taken into consideration, as previously described [4], [7].

We, thus, admitted that the cationic form of procaine can penetrate into the laminar (cationitic) micellae of the external membrane layer instead of the structuring $Ca^{2+}$ [5], [18], leading to an increase in their resistance to the action of destructuring agents ($K^+$). Replacing $Ca^{2+}$, procaine determines also a diminution, respectively a slowing down of the external high $K^+$ induced depolarization. This explains, on the other hand, some aspects of the specific procaine effect upon the action potential, expressed by : a decrease in the speed of the upward phase of the action potential, a reduction of its amplitude, a decrease in the potassium conductance and an increase in the length of the downward phase of the action potential [9], [11], [15], [17]. But electrically charged procaine also acts upon the globular micellae of the external layer, changing them by phase transition into laminar micellae, accompanied by a membrane potential increase, exactly as $Ca^{2+}$ in excess. In the already depolarized membrane, characterized by globular micellae, procaine added to the medium leads to the replacement of potassium and to a partial repolarization.

In our experiments, in which procaine (2.5 mM) is added to the medium at the same time with high-$K^+$, at the level of the external membrane layer three simultaneous processes take place : on the one hand, a transition from laminar to globular micellae induced by $K^+$, with a decrease in the membrane potential, on the other hand, a transition from globular to laminar micellae, and an increase in the own resistance of the latter, induced by procaine, both anesthetic effects leading to a membrane potential increase. In this situation, the general effect of procaine is a strong slowing down of the $K^+$ induced depolarization, at pH = 7.2 (Fig. 1—A) and a slowing down and decrease in its amplitude at pH = 6 (Fig. 2—A). In a previous paper [18] we could demonstrate, at this pH, a complete reduction of the membrane depolarization in $Ca^{2+}$-free Ringer, due to procaine.

In order to understand these effects we have to keep in mind the fact that, generally speaking, at pH = 7.2, the interaction efficiency between procaine and $K^+$ is smaller than the one between $Ca^{2+}$ and $K^+$ [1], [7]. However, at pH = 6, although the $K^+$ depolarization is more intense, the effect of procaine is also stronger. This may be explained if we consider the acid pH induced modifications at the level of the lipid membrane structures [19], which favour the action of procaine, the increase of its cationic form concentration and its capacity to simulate the role played by $Ca^{2+}$ [18] under such conditions. As a result of these acid pH conditions, which favour the action of the procaine cationic form, an important number of laminar micellae, which bind procaine, are no longer implicated in the phase transition. Thus, the depolarization amplitude remains smaller.

When fibres are washed with normal Ringer, membrane repolarization takes place, which is, usually, much slower (Figs. 1—A and 2—A) than in control experiments (Figs. 1—B and 2—B) indicating the persistence of the anesthetic within the membrane. The reduction in the repolarization speed, however, is much stronglier expressed at pH = 7.2 than at pH = 6. Similar effects were also observed when procaine was added after the membrane has been already depolarized by $K^+$ [6].



The strong slowing down of the repolarization in normal alkaline medium cannot be attributed to the cationic procaine, since this tends to organize the phospholipidic micellae into the laminar form. If we take into account that in our experiments the depolarization, corresponding to the $K^+$ concentration used, has totally accomplished, although with a delay we have to admit a complete transformation of the laminar micellae into globular ones (corresponding to the $K^+$ concentration). This means that procaine, which opposes the repolarization, is associated with these micellar structures and thus its interaction with the membrane is not based on an interference with the role played by $Ca^{2+}$. Such an interaction of procaine with the globular (anionitic) membrane structures forms the base of the stabilizing effect of the uncharged form of the anesthetic upon the internal layer (predominantly cationitic). This explains how the membrane repolarization takes place in alkaline medium. The external layer of the depolarized membrane is, from a structural and electrical point of view, in the same state as the internal layer under normal conditions. Neutral procaine, which, at pH = 7.2, is present in a fairly high concentration in the medium [8], stabilizes effectively the depolarized membrane, the rebinding of $Ca^{2+}$ taking place very slowly, while the anesthetic is gradually replaced from the membrane.

The membrane repolarization at pH = 6 is however quicker, since on the one hand, procaine exists almost entirely in its cationic form [8], and on the other hand, the phospholipid substratum itself contains a relatively smaller number of globular micellae. In this instance, however, there exists an antagonism between the electrically charged procaine and $Ca^{2+}$.

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# PARASITIC *HYMENOPTERA (BRACONIDAE)* OF THE LEPIDOPTEROUS PESTS OF ALFALFA CROPS

BY

MATILDA LĂCĂTUȘU and MIHAIL C. MATEIAȘ

Twenty-three Braconids were identified in parasitized hosts sampled in the area investigated and reared in the laboratory. Among these, *Orgilus pimpinellae* Niez., *Apanteles immunis* Hal. and *Apanteles pallipes* Reisch. are new species in the fauna of Romania.
Several species of Lepidoptera are new hosts to science for many of the Braconids presented.

The insect pests of alfalfa crops include many species of Lepidoptera, most of them being lately recognized as new potential pests [3], [5].

The natural limitation of insect pest population by parasites is worth studying since its effects are very important. As regards the Lepidopterous pests of alfalfa crops, a first step was already made and involves the identification of the parasites, of larvae and pupae belonging to the families *Braconidae, Encyrtidae, Eulophidae, Chalcididae, Ichneumonidae (Hymenoptera)* and *Larvaevoridae (Diptera)* [1], [2], [4], [6] [7].

The paper presents these investigations of several new species of Braconid parasites of the Lepidopterous larvae infesting the alfalfa crops in the Fundulea-Ilfov area, and described new biological aspects of its development.

## MATERIAL AND METHODS

The species of parasitic Braconids were obtained by rearing their hosts under controlled conditions in growth chambers from 1977 till 1979. The larvae were collected with the net in the alfalfa fields of the Research Institute for Cereals and Industrial Plants — Fundulea. They were introduced singly in plastic boxes having the lid covered with fine wire mesh and were fed with alfalfa.

The rearing conditions were as follows: 12 hours in the light at 23—24°C alternating with 12 hours in the dark at 19—20°C; relative humidity was maintained at 65—75%.

## RESULTS

Seven out of the 23 parasitic Braconides identified in 1975 — 1979 (table 1), namely *Chelonus annurus, Pelecystoma lutea, Apanteles lineola,*

*Apanteles congestus*, *Apanteles jugosus*, *Apanteles praepotens*, *Apanteles sesilis*, *Microplitis spinolae* and *Microplitis scrophulariae*, collected and

*Table 1*

Braconids obtained from Lepidopterous pests of alfalfa crops in the area of Fundulea-Ilfov between 1975 — 1979

| No. | Subfamily | Genus Species |
|---|---|---|
| 1 | Rhogadinae | *Rhogas testaceus* Spin. |
| 2 | Rhogadinae | *Pelecystoma lutea* Nees. |
| 3 | Euphorinae | *Meteorus rubens* Nees. |
| 4 | Mimagathinidae | *Orgilus pimpinellae* Niez. |
| 5 | Cheloninae | *Chelonus oculator* Panz. |
| 6 | Cheloninae | *Chelonus annulipes* Wesm. |
| 7 | Microgasterinae | *Microplitis mediator* Hal. |
| 8 | Microgasterinae | *Microplitis spinolae* Nees. |
| 9 | Microgasterinae | *Microplitis tuberculifera* Wesm. |
| 10 | Microgasterinae | *Microplitis scrophulariae* Szepl. |
| 11 | Microgasterinae | *Protomicroplitis scotica* Marschl. |
| 12 | Microgasterinae | *Apanteles ruficrus* Hal. |
| 13 | Microgasterinae | *Apanteles congestus* Nees. |
| 14 | Microgasterinae | *Apanteles sesilis* Ill. |
| 15 | Microgasterinae | *Apanteles vanessae* Reinh. |
| 16 | Microgasterinae | *Apanteles juniperatae* Bouché. |
| 17 | Microgasterinae | *Apanteles praepotens* Hal. |
| 18 | Microgasterinae | *Apanteles jugosus* Lyle. |
| 19 | Microgasterinae | *Apanteles pieridis* Bouché. |
| 20 | Microgasterinae | *Apanteles lineola* Curt. |
| 21 | Microgasterinae | *Apanteles immunis* Hal. |
| 22 | Microgasterinae | *Apanteles inclusus* Ratz. |
| 23 | Microgasterinae | *Apanteles pallipes* Reisch. |

determined in 1975 — 1976, have been already reported [1]. Consequently, these species will not be further discussed unless their hosts are new.

— Subfamily *Rhogadinae*

1. *Rhogas testaceus* Spin. 1 ♂ and 1 ♀ obtained on 25.07. 1977 from larvae of *Eupithecia centaureata* Den. et Schiff. *(Geometridae)*. The cocoon is elongated, cylindrical, silver-grey, with small black dots (Fig. 1.). This is a polyphagous species developing in larvae of Geometrids, Noctuids, etc.; the host is new to science.

— Subfamily *Euphorinae*

2. *Meteorus rubens* Nees. Eighteen individuals were obtained on 24.10. 1979 from a single larva of *Scotia segetum* Den. et Schiff. The cocoon is oval-shaped, light brown and rather tapered at the hind end (Fig. 2). This parasite is known to occur in the above mentioned Lepidoptera and in other Noctuids [8].

— Subfamily *Mimagathidinae*

3. *Orgilus pimpinellae* Niez. 1 ♂ and 1 ♀ obtained from larvae of *Semiothisa clathrata* L. *(Geometridae)* on 5.08 1978. The cocoon is oval, elongated, grey (Fig. 3). Single parasite of Geometrid larvae; the host



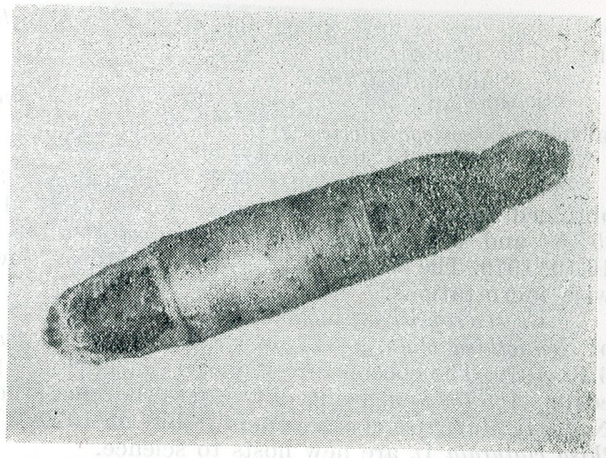

Fig. 1. — Cocoon of *Rhogas testaceus* Spin.

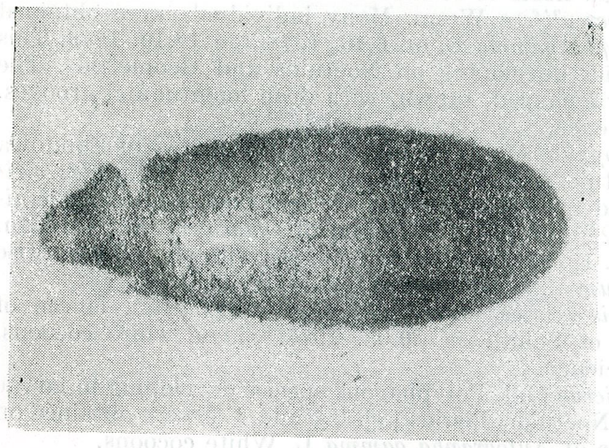

Fig. 2. — Cocoon of *Meteorus rubens* Nees.

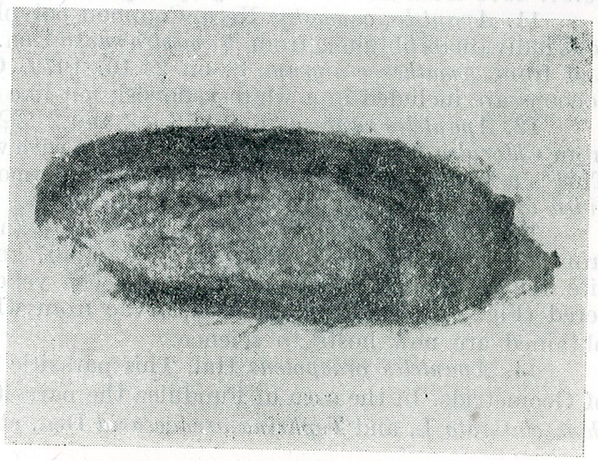

Fig. 3. — Cocoon of *Orgilus pimpinellae* Niez.

of this species is new to science. *Orgilus pimpinellae* is a new species in the fauna of Romania.

— Subfamily *Cheloninae*

4. *Chelonus oculator* Panz. 1 ♂ and 1 ♀ were obtained from one larva of *Loxostege sticticalis* L. on 12.09. 1979.

— Subfamily *Microgasterinae*

5. *Microplitis mediator* Hal. 5 individuals (3 ♂♂ and 2 ♀♀) were obtained from *Autographa gamma* L. on 17.07. 1978 and 9 individuals (5 ♂♂ and 4 ♀♀) from *Mamestra suasa* Den. et Schiff. *(Noctuidae)* on 16.10. 1979. The cocoon (Fig. 4) is light brown, downy, with slight lengthwise incrustations.

6. *Microplitis spinolae* Nees. 2 ♂♂ and 4 ♀♀ obtained from larvae of *Semiothisa clathrata* L. on 14.08. 1979 and *Autographa gamma* L. on 6.10. 1979. The cocoons are single, greenish (Fig. 5). The parasite is reported on *Agrotis spp.* being obtained in the area studied from larvae of the Noctuid *Mamestra suasa* Den. et Schiff. in 1976 [1]. The larvae of *Semiothisa clathrata* L. are new hosts to science.

7. *Microplitis tuberculifera* Wesm. Many individuals were obtained from larvae of *Autographa gamma* L. on 6.10. 1978 and 16.10. 1979. This is a polyphagous species developing on Noctuids and Geometrids. The cocoons (Fig. 6) are oval-shaped, brown, with deep longitudinal grooves. The host is new to science.

8. *Microplitis scrophulariae* Szepl. Reported in the area of Fundulea as parasite of the Noctuid species *Ectypa glyphica* L. and *Chloridea viriplaca* Hfn. [1]. It was identified as parasite of the larvae of *Amathes c-nigrum* L. (3 ♂♂ and 2 ♀♀) on 12.10. 1978 and of *Chloridea viriplaca* Hfn. (1 ♂ and 1 ♀) on 10.09. 1979. Single cocoon (Fig. 7) with slight lengthwise stripes. *Amathes c-nigrum* L. is a new host to science.

9. *Protomicroplitis scotica* Marsh. 3 ♂♂ obtained from larvae of *Mamestra suasa* Den. et Schiff. on 30.08. 1977. Single white cocoons. The host is new to science.

10. *Apanteles ruficrus* Hal. Polyphagous species developing in larvae of Tortricids, Pyralids, Noctuids, Pierids [8]. 1 ♂ and 1 ♀ were obtained on 29.07. 1979 from larvae of *Autographa gamma* L. White cocoons.

11. *Apanteles congestus* Nees. Common polyphagous parasite. Numerous individuals obtained from *Mamestra suasa* Den. et Schiff. on 18.10.1979 and from *Amathes c-nigrum* L. on 24.10. 1979. Gregarious species; the cocoons are included in a white-yellowish felt-like mass (Fig. 8).

12. *Apanteles vanessae* Reinh. 3 ♂♂ and 2 ♀♀ obtained on 28.09.1979 from *Chloridea viriplaca* Hfn. Social parasite with white-yellowish cocoons. This is a host new to science the species being reported on Noctuids *(Chloridea armigera* Hb.) and Nimphalids [8].

13. *Apanteles juniperatae* Bouché. 6 ♂♂ and 6 ♀♀ were obtained from larvae of *Semiothisa clathrata* L. on 13.07 and 11.09.1978 the parasite being specific to Geometrid larvae. The yellowish cocoons are clustered (Fig. 9). The Lepidopterous larvae from which the parasites were obtained are new hosts to science.

14. *Apanteles praepotens* Hal. This parasitic species is characteristic of Geometrids. In the area of Fundulea the parasite was found on *Semiothisa clathrata* L. and *Tephrina arenacearia* Den. et Schiff. [4]. Many indi-



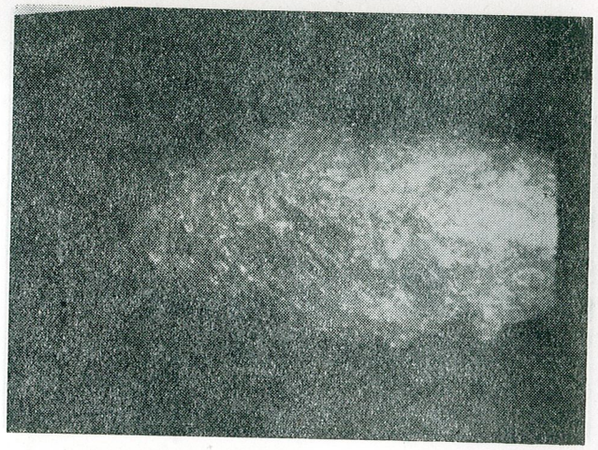

Fig. 4. — Cocoon of *Microplitis mediator* Hal.

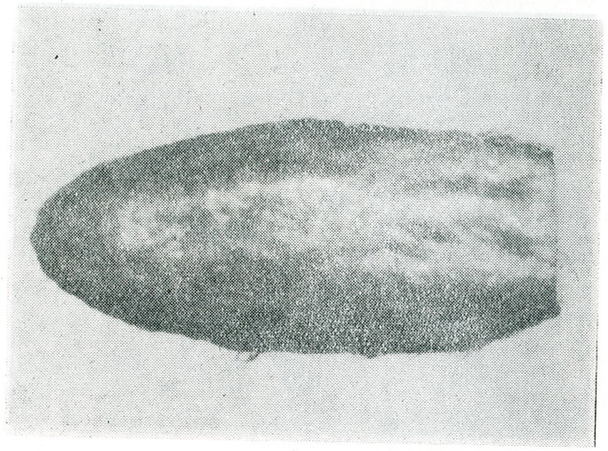

Fig. 5. — Cocoon of *Microplitis spinolae* Nees.

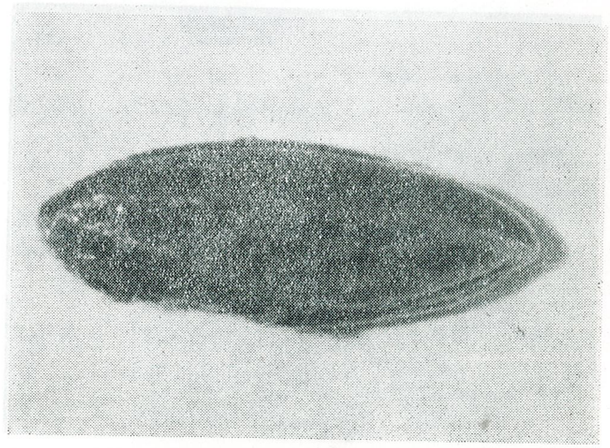

Fig. 6. — Cocoon of *Microplitis tuberculifera* Wesm.

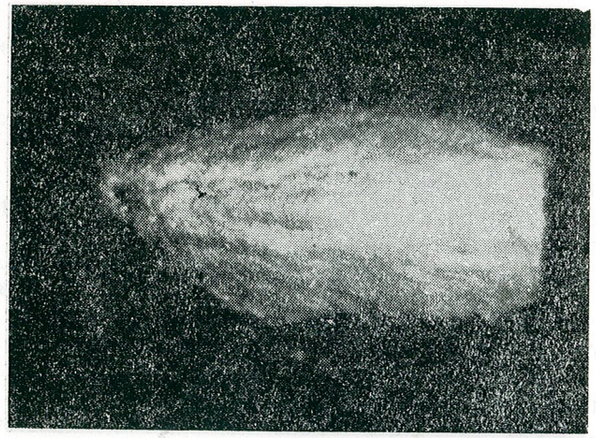

Fig. 7. — Cocoon of *Microplitis scrophulariae* Szepl.

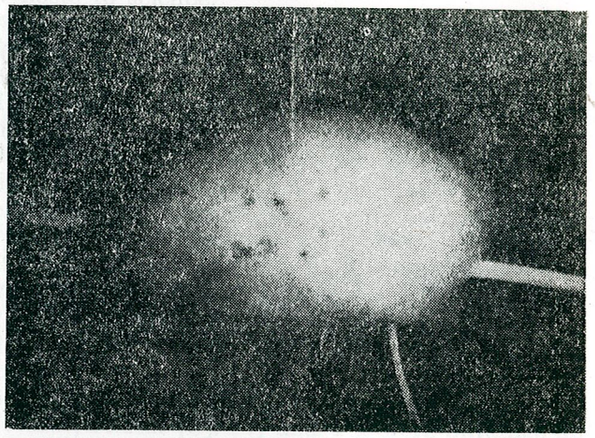

Fig. 8. — Cocoon of *Apanteles congestus* Nees.

Fig. 9. — Cocoon of *Apanteles iuniperatae* Bouché



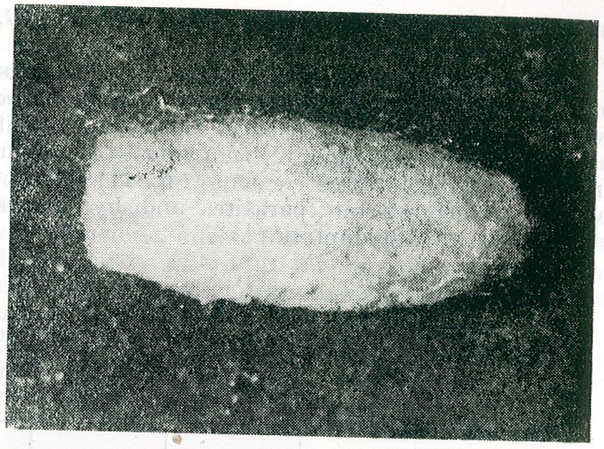

Fig. 10. — Cocoon of *Apanteles praepotens* Hal.

Fig. 11. — Cocoon of *Apanteles pieridis* Bouché

Fig. 12. — Cocoon of *Apanteles immunis* Hal.

viduals of this parasite appeared in October 1977, 1978 and 1979 on the above-mentioned hosts. The cocoon is shown in Fig. 10.

15. *Apanteles pieridis* Bouché. The cocoons of the parasites were obtained in several cases from larvae of *Colias croceus* Fourcr. *(Pieridae)* which is a host new to science. Both the parasite (♂♂ and ♀♀) and a hyperparasitic Ichneumonid belonging to the genus *Mesochorus* appeared from the white clustered cocoons (Fig. 11). Thus, table 2 shows the number of Hymenopterous parasites and hyperparasites, obtained from a single larva of Lepidoptera.

*Table 2*

| Date | No.of cocoons obtained | No. of Hymenoptera emerged | |
|---|---|---|---|
| | | *Apanteles pieridis* | *Mesochorus* spp. |
| 8.X. 1979 | 10 | 4 | 6 |
| 12.X. 1979 | 7 | 2 | 5 |
| 16.X. 1979 | 5 | 1 | 4 |
| 20.X. 1979 | 9 | 4 | 5 |
| 27.X. 1979 | 12 | 5 | 7 |
| Total | 43 | 16 | 27 |

It can be seen that in October 1979 the parasitic species *Apanteles pieridis* was in turn parasitized (in a proportion of 62. 79%) by the hyperparasitic Ichneumonid.

16. *Apanteles immunis* Hal. Eight parasites (4 ♂♂ and 4 ♀♀) and 6 parasites (2 ♂♂ and 4 ♀♀) were obtained on 27.09. 1978 and 5.10.1979, respectively, from the Geometrids *Semiothisa clathrata* L. and *Tephrina arenacearia* Den. et Schiff. The cocoons are white, (Fig. 12) arranged in clusters of 5—6 each [8]. The species is polyphagous and parasitizes the larvae of Tortricids and Geometrids. Both hosts are new to science and the parásite is a new species in the fauna of Romania.

17. *Apanteles inclusus* Ratz. On 15.08. 1977 2 ♂♂ and 2♀♀ were obtained from larvae of *Mamestra suasa* Den. et Schiff. The cocoons are with a yellowish hue, clustered.

18. *Apanteles pallipes* Reisch. Many individuals were obtained from larvae of *Autographa gamma* L. on 6.10. 1979. Clustered cocoons are covered by a silky coat. This parasitic species is new in the fauna of Romania.

## CONCLUSIONS

1. Between 1975 and 1979, 23 species of Braconids belonging to 6 subfamilies were obtained at Fundulea-Ilfov by rearing in the laboratory the parasitized hosts.

2. Three parasitic Braconids, i.e. *Orgilus pimpinellae* Niez., *Apanteles immunis* Hal., *Apanteles pallipes* Reisch., are new for the fauna of Romania, and 14 species (obtained between 1977 and 1979) have not been yet reported in the investigated area.



3. The Lepidoptera *Semiothisa clathrata* L., *Tephrina arenacearia* Den. et Schiff., *Eupithecia centaureata* Den. et Schiff. *(Geometridae)*, *Autographa gamma* L., *Mamestra suasa* Den. et Schiff., *Amathes c-nigrum* L., *Chloridea viriplaca* Hfn. *(Noctuidea)* and *Colias croceus* Fourcr. *(Pieridae)* are still not cited in the literature on most of the Braconids identified, as being new hosts to science.

4. Five species of Braconids infested the *Semiothisa clathrata* L. larvae. This biological peculiarity is useful for the biological control, because in natural conditions it is possible to maintain the parasite population for a longer time.

5. Some of the parasites are not specific, being characterized by a highly polyphagous habit *(Apanteles congestus)*, and others *(Apanteles pieridis)* are specialized to a limited host range.

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M.A. IONESCU, *Termitele (The Termites)*, Edit. Academiei, București, 1980, 148 pp, 37 figs.

The difficult task of presenting the Termites spread all over the world is remarkably accomplished by this monographic work elaborated by an entomologist of national and international reputation.

Termites, social insects, represent a well-delimited group by both their marked polymorphism and their way of building up nests. From the 104 genera with 1709 species of the order *Isoptera* spread throughout the world, it is a single species, i.e. *Reticulitermes lucifugus* (Kollar) of the family *Rhinotermitidae* that occurs in our country. The species has been cited since 1905 by Kempry, detailed studies being dedicated to it between 1932—1978 by the author of the above mentioned work.

After briefly dealing with the history of the research, the monograph is separated in chapters dwelling upon the systematics and nomenclature, paleontology and phylogeny, geographic distribution, ontogenetic development, morphology, anatomy and physiology, ecology and ethology, behaviour and economic importance of the Termites.

The references consulted are selectively presented summing up 175 titles. The work ends by a taxonomic index and a summary in English.

We heartedly recommend this work to both entomologists and public at large interested in learning the misteries of this attractive group of insects.

Both as content and graphic presentation are concerned, this book is an achievement.

It is a pity that the book was issued in rather few copies, as it is the only work of its kind in Romania which has greatly stimulated the interest of the reading public.

*M.C. Mateiaș*