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SUR LES CARACTÈRES SEXUELS SECONDAIRES DE LA NYMPHE D'*ABROLOPHUS IACOBI* (ERYTHRAEIDAE) ET LEUR IMPORTANCE TAXONOMIQUE

PAR

Z. FEIDER

The perigamic character of the nymph of *Albrolophus iacobi* n.sp. is described at the level of the same organs as revealed in the adult. As far as the perigamic character is concerned there are two types of nymphae, namely: "paidicoperigamic" and "synchronal perigamic", with taxonomical importance.

Dans un autre article nous avons décrit l'adulte (♂ et ♀) de l'espèce *Abrolophus iacobi* n.sp. de la famille *Erythraeidae* Robineau-Desvoidy, 1894 (**3**), qui est un acarien prédateur des pucerons des plantes. À cette occasion nous avons mis en évidence les caractères sexuels secondaires (caractères périgamiques, D a v i t a s h i l i, 1961) de l'espèce.

Dans le présent article nous décrivons la nymphe de la même espèce, chez laquelle nous avons trouvé des caractères périgamiques au niveau des mêmes organes que chez l'adulte.

Les dimensions, en microns, des nymphes sont notées dans le tableau 1.

Tableau 1

Les dimensions, en microns, des nymphes d'*Abrolophus iacobi*

Organes	n.♀	n.♂	Organes		n.♀	n.♂
Idiosome	650/475	550/350	Pattes	I	725	700
Gnathosome	165/100	200/150		II	470	460
Aréole I	45/40	30/40		III	525	500
ASB I	30	20		IV	825	770
PSB I	150	135	IP		2545	2430
SB-SB	115	120	Tarse	I	85/65	95/45
ASB II	175	130		II	60	60
PSB II	25	25		III	60	65
S I	10	12		IV	75	60
S II	12	10	Tibia	I	120	120
Aréole II	25/25	25/15		II	95	115
Palpe	130/50	150/40		III	120	85
Palpotarse	20/15	18/12		IV	180	150
Chélicère	350	400	Uropore		40/20	46/26

La nymphe est dépourvue d'orifice génital (fig. 1), fait auquel nous attribuons une importance particulière dans la caractérisation du groupe *Erythraeoidea*.

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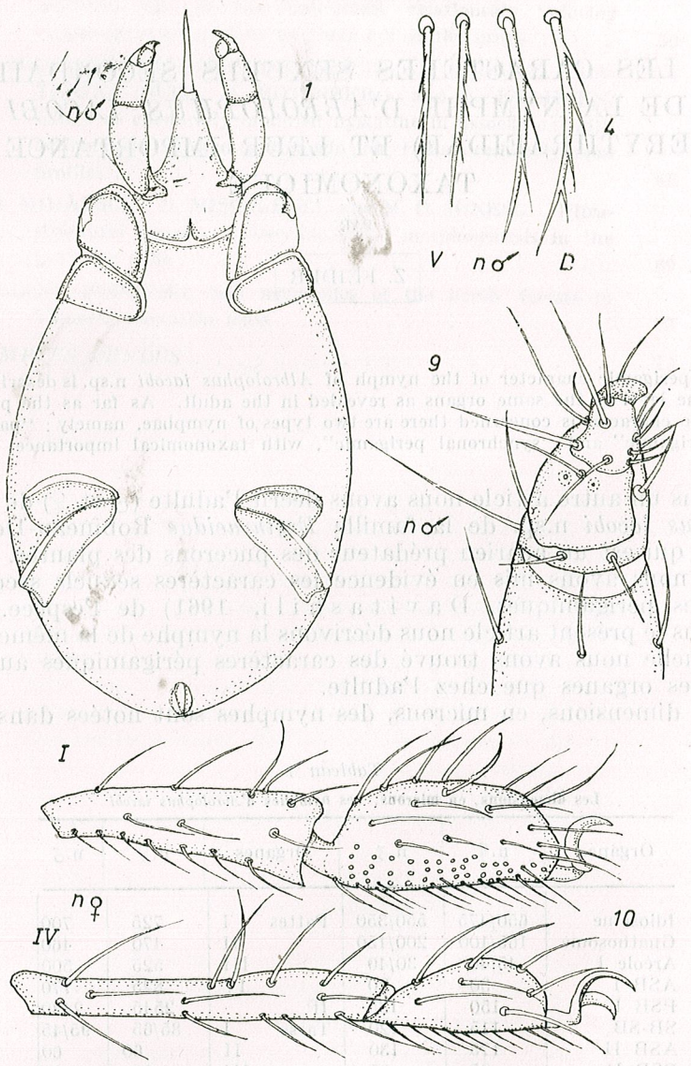

Fig. 1. — Face ventrale ; Fig. 4, D. Poils dorsaux, V. Poils ventraux ; Fig. 9. — Extrémité du palpe ; Fig. 10. I, Patte I, IV, Patte IV.

Sur la partie dorsale de la nymphe on observe la crête métopique dont l'extrémité antérieure est munie d'une aréole triangulaire, dont les bords latéraux sont droits chez la nymphe ♀ (fig. 2) ou profondément

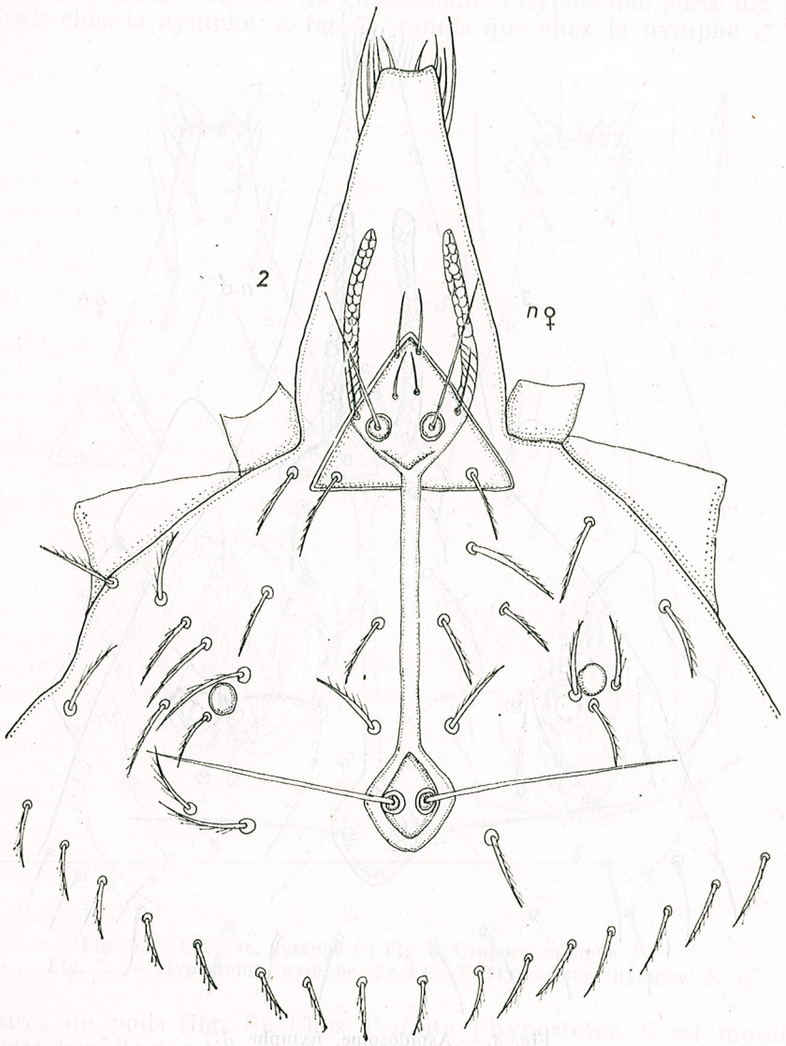

Fig. 2. — Aspidosome, nymphe ♀.

excavés chez la nymphe ♂ (fig. 3). Les aréoles des nymphes ressemblent aux aréoles des sexes adultes. Les poils paracristaux (de tous côtés de la crête métopique) sont en nombre de 14 paires chez la nymphe ♀ et de huit paires chez la nymphe ♂. De même, les poils paracristaux des adultes sont en nombre de 40 paires chez la ♀ et de 49 chez le ♂. Le dia-

mètre de la cornée est plus grand chez la nymphe ♂ et chez le ♂. Les poils de l'idiosome des nymphes ont 3—4 barbules et le rachis plus fort à la partie dorsale (fig. 4), comme chez les adultes.

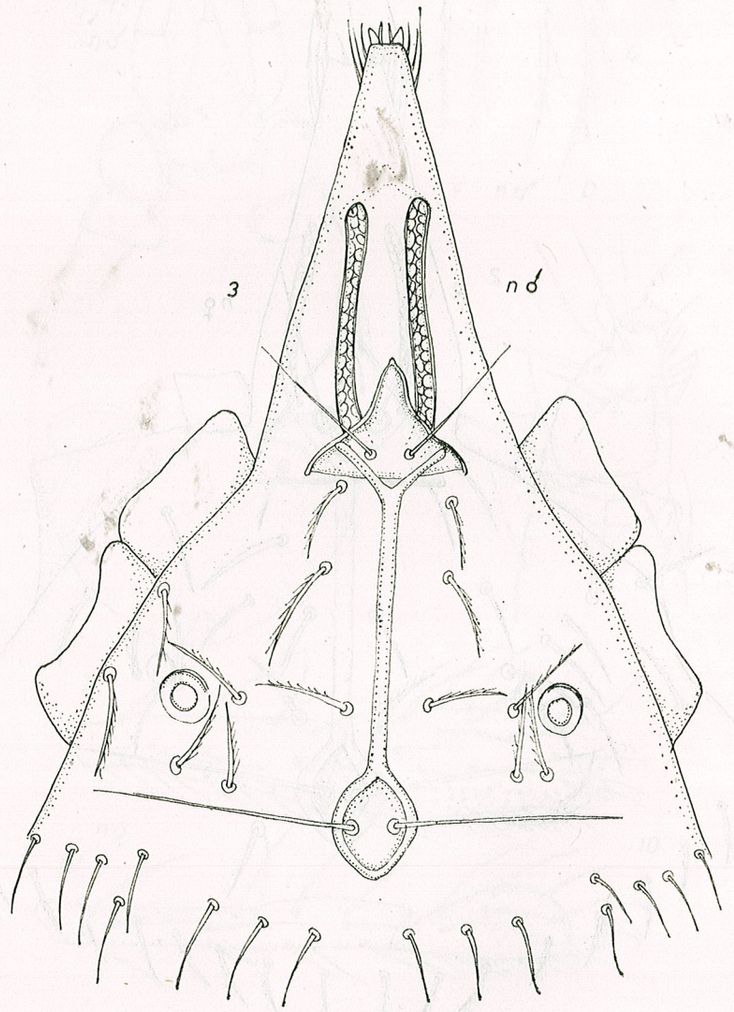

Fig. 3. — Aspidosome, nymphe ♂.

Sur la face ventrale, l'uropore de la nymphe a une position terminale et il est fermé par deux valves latérales, qui portent un nombre fixe de poils selon le sexe, à savoir trois paires chez la nymphe ♀ (fig. 5) et quatre paires chez la nymphe ♂ (fig. 6). De même chez l'adulte le nombre de poils uroporaux est fixé, c'est-à-dire cinq paires chez la ♀ et six paires chez le ♂. Comme chez le phalange *Trombidia* Feider, 1959 (**1**), il s'agit

d'un uropore hétérouropore, différent chez les deux sexes, et du type macrandre, c'est-à-dire plus riche en poils chez le mâle. D'après l'uropore on peut facilement reconnaître le sexe de la nymphe ou de l'adulte.

Sur la partie ventrale du gnathosome, l'hypostome porte dix paires de poils chez la nymphe ♀(fig. 7), tandis que chez la nymphe ♂ il y a

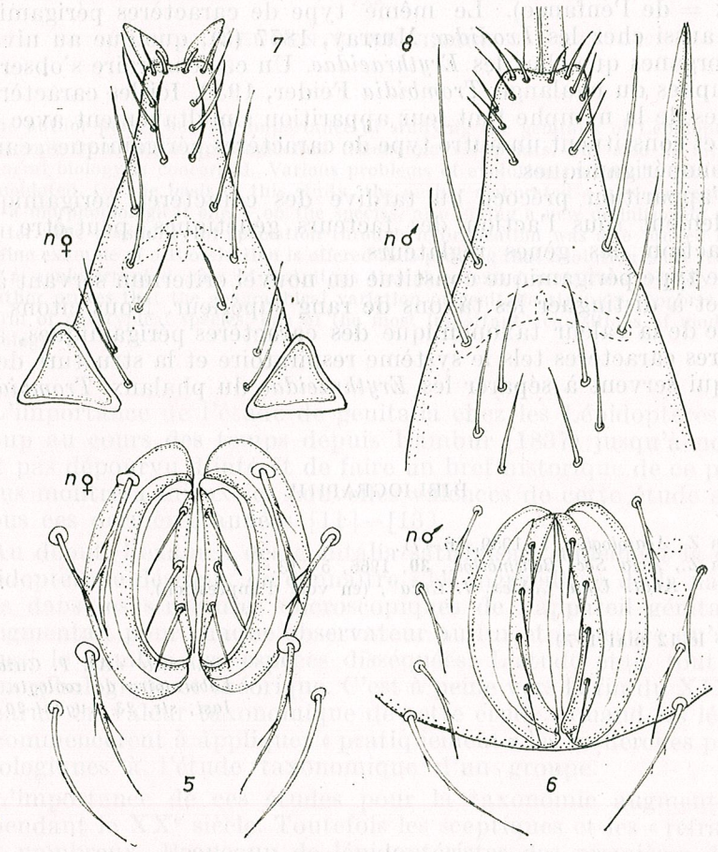

Fig. 5. — Uropore, nymphe ♀; Fig. 6, Uropore, nymphe ♂.
Fig. 7. — Hypostome, nymphe ♀; Fig. 8, Hypostome, nymphe ♂.

12 paires de poils (fig. 8). Chez l'adulte l'hypostome ♀ est munie de 19 paires de poils, tandis que l'hypostome ♂ a seulement 15 paires de poils. Tant chez la nymphe ♀ que chez la ♀ adulte les poils apicaux internes de l'hypostome sont émoussés, tandis que la nymphe ♂ et le ♂ ont les poils apicaux aigus. Le palpotarse est muni de 11 paires de poils lisses chez les nymphes (fig. 9) et de 21 paires de poils lisses chez les adultes.

Les pattes des nymphes, comme chez les adultes, portent des poils plus longs à la partie dorsale, mais plus rares que sur la face ventrale (fig. 10).

Chez la nymphe d'*Abrolophus iacobi*, comme chez les nymphes des autres espèces de la famille *Erythraeidae*, les caractères périgamiques se trouvent au niveau des mêmes organes que chez les adultes. Ces caractères chez la nymphe font leur apparition avant le développement de l'orifice génital et peuvent être considérés comme des caractères différentiels précoces entre les deux sexes (paidicopérigamiques du grec παιδικός = de l'enfance). Le même type de caractères périgamiques se trouve aussi chez les *Ixodidae* Murray, 1877 **(2)**, quoique au niveau des autres organes que chez les *Erythraeidae*. Un cas contraire s'observe chez les nymphes du phalange *Trombidia* Feider, 1959. Ici les caractères périgamiques de la nymphe font leur apparition simultanément avec l'orifice génital et constituent un autre type de caractères périgamiques, caractères synchronopérigamiques.

L'apparition précoce ou tardive des caractères périgamiques est probablement sous l'action des facteurs génétiques, peut-être sont-ils sous l'action des gènes réglateurs.

Le type périgamique constitue un nouvel criterium servant à caractériser et à distinguer les taxons de rang supérieur. Nous citons un seul exemple de la valeur taxonomique des caractères périgamiques, associés à d'autres caractères tels le système respiratoire et la structure des chélicères, qui servent à séparer les *Erythraeidae* du phalanx *Trombidia*.

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LES GENITALIA DES LÉPIDOPTÈRES À L'APPUI DE L'ÉVOLUTIONNISME

PAR

EUGEN V. NICULESCU

The author points out the importance of studying the genitalia of Lepidoptera not only from the morphologic and taxonomic viewpoints, but also as far as general biology is concerned. Various problems of evolutionism may be this way elucidated. On the basis of this study, the author elaborated a new conception — a morphobiological one — on the species, also giving a new definition of this latter. The possibility of speciation through hybridization was also proved and a fine example of orthoselection is offered thus proving that evolution is the result of a noninterrupted raw of fortuitous though oriented mutations. Finally the author proves that the geographical variation of genitalia strongly supports the term of polytypical species, one of the most important acquisitions of modern biology.

L'importance de l'étude de genitalia chez les Lépidoptères a varié beaucoup au cours des temps depuis Rambur (1837) jusqu'à nos jours. Il n'est pas dépourvu d'intérêt de faire un bref historique de ce problème qui nous montrera mieux les nouvelles valences de cette étude signalées par nous ces dernières années [11]–[13].

Au début, l'examen des genitalia satisfaisait seulement la curiosité du lépidoptériste désireux de connaître « les merveilles » de la nature exprimées dans les structures microscopiques de l'appareil génital. L'extase augmentait pour chaque observateur au fur et à mesure qu'augmentait aussi le nombre des espèces disséquées. L'étude était tout simplement morphologique et théorique. C'est à peine vers la fin du XIX^{e} siècle qu'apparut la valeur taxonomique de cette étude, quand les lépidoptéristes commencèrent à appliquer « pratiquement » les recherches purement morphologiques à l'étude taxonomique d'un groupe.

L'importance de ces études pour la taxonomie augmentait sans cesse pendant le XX^{e} siècle. Toutefois les sceptiques et les « réfractaires » étaient nombreux. Beaucoup de lépidoptéristes des premières décennies du XX^{e} siècle ignoraient totalement l'étude des genitalia et certains étaient catégoriquement réfractaires. Ainsi, le grand lépidoptériste français Ch. Oberthür écrivait en 1904 : « Les genitalia ont comme caractère spécifique la même valeur que tous les autres caractères, mais rien de plus ». Cette opinion erronée a persisté, inaltérée, jusqu'à nos jours, quoique le contraire ait été démontré par un grand nombre de lépidoptéristes. Nous avons prouvé [10], [11], [12], [15] que la valeur taxonomique des genitalia dépasse de beaucoup celle de tous les autres caractères ce qui nous a déterminé à considérer l'armure génitale comme un « phénomène morphologique ».

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On a exprimé l'opinion, également erronée, selon laquelle l'étude exagérée des genitalia aboutit à la conception typologique de l'espèce! Quoiqu'elle ne soit partagée par aucun lépidoptériste, toutefois pour qu'elle ne soit pas reprise aussi par d'autres zoologistes, il faut préciser que la conception typologique de l'espèce est une conception fixiste considérant l'espèce comme immuable, tandis que l'étude du « phénomène morphologique » est par contre du plus pur évolutionnisme.

La même idée a été exprimée aussi par le prof. Henri Descimon [3] qui dit :

« Creationniste, le concept typologique est évidemment aussi fixiste : les espèces sont là une fois pour toutes, œuvres du Créateur ou, pour les athées, de la Nature — c'est ainsi ». De même M. Guillaumin et H. Descimon [4] affirment : « A l'heure actuelle, le concept typologique est encore appliqué, par les systématiciens qui considèrent qu'à l'échelle du temps de nos observations les espèces évoluent peu et paraissent stables ».

Et maintenant montrons quelles sont les nouvelles valences de ce phénomène.

1. En étudiant d'une part le critère biologique de l'isolement reproductif et d'autre part la valeur taxonomique des genitalia, nous avons constaté que le critère mixiologique est un bon critère d'espèce, mais par contre qu'il est déficient comme critère interspécifique, c'est-à-dire qu'il ne nous aide pas du tout à délimiter les espèces entre elles et à les identifier [15]. A cette fin l'armure génitale est un critère idéal [7], [9], [13], [14].

Vu que de nombreuses *bonae species* sont interfertiles (au lieu d'être interstériles comme postule le critère mixiologique), vu aussi le fait que l'isolement des espèces n'est pas seulement biologique mais aussi morphologique [1], écologique, génétique, etc. nous avons considéré que le critère d'espèce et interspécifique le meilleur n'était le critère biologique mais le critère morpho-biologique [8],[9]. Cette nouvelle conception, concrétisée dans une nouvelle définition sur l'espèce [8] a commencé d'attirer l'atention des spécialistes de l'étranger. Ainsi E. Sabariego [17] dit dans un travail récent : « Nos referimos al criterio *morfo-biologico* de tan general aceptation y elaborado por biologos de todo tiempo y de todo et mundo, desde Linné a Niculescu ». Ainsi, l'étude des genitalia chez les Lépidoptères nous a conduit à l'élaboration d'un nouveau concept d'espèce.

2. En ce qui concerne l'étude, « exagérée » des genitalia, il faut préciser qu'elle n'a aucun rapport avec la conception typologique. Par contre cette étude nous a montré que chez certaines espèces il y a dans les genitalia une variation géographique Ainsi la notion d'espèce polytypique trouve son illustration et sa justification aussi dans l'étude détaillée des genitalia.

[1] Cela est admis même par E. Mayr [3] le promoteur du concept biologique de l'espèce quand il dit : « L'isolement reproductif, les différences écologiques et la *distinction morphologique* (notre soulignement) sont les trois propriétés les plus caractéristiques de l'espèce ». Toujours Mayr reconnait qu'il y a certaines espèces différenciées au point de vue morphologique, mais ne sont pas encore isolées au point de vue reproductif. Mais les disciples sont, parfois, plus orthodoxes que le maître puisque l'un de ceux-ci dit catégoriquement : « Nous ne pouvons affirmer que deux formes voisines sont devenues espèces distinctes que si l'on constate l'isolement reproductif dans la nature ».

3. L'étude de l'armure génitale chez *Eurytides helios* (Papilionidae) comparativement à celle des espèces *E. telesilaus* et *E. protesilaus* nous a prouvé la possibilité d'une spéciation par hybridation — phénomène contesté (chez les animaux) par E. Mayr [5]. Récemment C. Nagy a affirmé dans un travail [6] que l'« hybridation entre les espèces voisines joue un rôle important dans le processus de la spéciation chez les Hyménoptères parasites ».

4. L'examen d'une grande série d'espèces dans le genre *Papilio* [11], [14] nous a prouvé que l'armure génitale présente chez ces espèces, un bel exemple *d'orthosélection* et que les sclérites dont il s'agit nous montrent que les mutations qui les ont produit sont orientées (*mutations convergentes*). L'étude minutieuse des genitalia chez les *Papilionidae exotiques* [13] nous a toujours montré l'existence de mutations fortuites (*mutations divergentes*). Ainsi, nous avons établi que l'*Évolution est le résultat d'une suite ininterrompue de mutations fortuites et mutations orientées.*

5. L'étude des genitalia chez les *Papilionidae* nous a conduit à l'élaboration d'une nouvelle notion, celle de *valence morphologique* des genres et des termes nouveaux *euryvalent*, *oligovalent* et *stenovalent* [12].

Par conséquent, l'étude de l'armure génitale est de la plus grande portée scientifique non seulement pour la taxonomie et la phylogénie des Lépidoptères, mais aussi pour la biologie générale en nous aidant à éclaircir divers problèmes de l'Évolutionnisme. Nous sommes d'avis que l'isolement morphologique des genitalia, différents d'une espèce à l'autre, est un phénomène réel dans la nature. Nous rappelons à cette occasion que le phénomène de l'isolement morphologique a été entrevu aussi par le savant E. Racovitza. Il écrit dans un de ses travaux où il parle du rôle de l'isolement dans la spéciation [16] : « Le cas est autrement quand une colonie de variants ou même de non-variants, est isolée de l'espèce mère géographiquement par des obstacles physiques (montagnes, mers, rivières, déserts, etc.) ou physiologiquement par modification de l'appareil copulateur ». Le « muséographe » s'il sait« voir » et interpréter justement ce qu'il a vu fait une bonne œuvre évolutionniste, même s'il étudie un matériel mort. Est-ce que l'œuvre des paléontologistes qui étudient seulement du matériel mort et fossilisé n'est pas une œuvre évolutionniste?

Mais la critique acerbe du critère morphologique est du domaine du passé. Actuellement les adeptes du concept biologique cèdent pas à pas et sont de plus en plus conciliants. Probablement nos critiques les ont-ils poussés à la médiation lorsque nous nous sommes montrés étonnés du fait qu'ils critiquent vivement le critère morphologique quand ils théorisent, mais l'utilisent à profusion quand ils travaillent en taxonomistes !

Dans certains travaux récents on peut lire les lignes suivantes : « L'isolement reproductif peut être détecté le plus facilement chez les oiseaux pendant qu'ils nichent, puis, chez une série de mammifères, insectes ou araignées. Chez d'autres groupes d'organismes, l'isolement reproductif dans la nature est difficile ou même impossible à constater dans l'état actuel de nos connaissances. Pour ces groupes le systématicien, même s'il est adepte du concept biologique de l'espèce sera obligé dans sa pratique taxonomique à délimiter les espèces *exclusivement selon les*

critères morphologiques » (le soulignement est le nôtre). Mais ces « d'autres groupes » représentent plus de 89% du total des espèces animales ! Si plus de 98% des espèces sont délimitées et identifiées selon les critères morphologiques peut-on encore dire que le critère morphologique est déficient et « vulnérable » ? Voici donc reconnue la justesse de nos idées après vingt années de « luttes ».

Notre point de vue est confirmé par P. Bănărescu [2] lorsqu'il dit : « Dans beaucoup de cas il y a des différences morphologiques tranchantes entre les espèces délimitées sur la base de l'isolement reproductif. Les adeptes du concept biologique et ceux du concept typologique de l'espèce reconnaissent dans la pratique courante les mêmes espèces. Les divergences apparaissent seulement dans le cas des espèces jumelles et celles polymorphes ». Vu que les espèces jumelles et polymorphes — où il y a des « divergences » — représentent une infime minorité à l'égard du reste des espèces où les deux concepts sont « convergents » et où le critère morphologique est, *enfin*, admis, la conclusion logique qui s'impose est donc la justesse de nos thèses. Certainement cette « réorganisation » de la conception de certains biologistes contemporains nous réjouit puisque la vérité scientifique a triomphé.

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LES ORGANES NÉURO-HÉMAUX PÉRISYMPATHIQUES DE *GRYLLOTALPA-GRYLLOTALPA* LATR. APRÈS LA CONSERVATION *IN VITRO*

PAR

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

This paper is a new contribution to the problem of tissue resistance during the grafting and preservation *in vitro*.
After 48 hours of preservation *in vitro* in a medium containing the hemolymph of the insects the pieces of which were prelevated from, the perisympathetic neurohemale organs keep their morphofunctionality : the perineurium is active and in the nervous fibres the neurosecretion is transferred and eliminated, as usual, in the interaxonal spaces.
The axo-axonal relations as well as the axo-glial ones are not changed.

Pour l'appréciation de la résistance tissulaire, nous avons tenu compte des critères suivants : le maintien de la structure et de l'activité du périneurium de l'organe neuro-hémal — en tant que barrière sensible intermédiaire entre le milieu de conservation et le tissu nerveux ; les aspects qui prouvent le maintien du transit normal interaxonal de la neurosécrétion et l'élimination spécifique des neurohormones ; les aspects qui prouvent la conservation des rapports morphologiques normaux entre les fibres nerveuses ainsi qu'entre les fibres nerveuses et les éléments névrogliques [1], [3], [5], [6].

MATÉRIEL ET MÉTHODE

En tant que milieu de conservation des organes neuro-hémaux de *Gryllotalpa*, nous avons utilisé même l'hémolymphe de l'insecte de laquelle on a prélevé les organes. L'hémolymphe a été diluée avec une solution physiologique (1 : 2) et sur le mélange on a ajouté une couche fine d'Ampicylline. Dans ce milieu on a gardé le système nerveux avec le complexe retrocérebral, pendant 48 heures. Dans ces expériences, les organes neuro-hémaux situés sur le trajet des nerfs segmentaires ont gardé *in vitro* des rapports normaux avec le système nerveux et un contact direct avec la lymphe, ces conditions se rapprochant de celles *in situ* en ce qui concerne les influences neuro-endocrines sur les organes neuro-hémaux. Après 48 heures on a effectué le fixage (pour la microscopie électronique) en glutaraldéhyde tamponnée, le post-fixage avec OsO_4, la déshydratation, l'inclusion en araldite et la coloration après Reynolds.

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RÈSULTATS ET DISCUSSIONS

Sous la neurilème homogène, non grossie après 48 heures de conservation, on a pu observer — tel que l'on avait déjà remarqué d'ailleurs [1], [5], [6] dans le périneurium de l'organe neuro-hémal — deux types de cellules épithéliales. Celles du premier type ont le noyau ovoïdal, faiblement chromatique, situé dans la moitié extérieure de la cellule (fig. 1). La membrane nucléaire présente un espace citernal de dimension uniforme. La chromatine constitue une couche très étroite qui se continue sur la partie intérieure de la citerne périnucléaire. De la chromatine périphérique s'en vont vers le nucléole des bandes fines et très rares de chromatine. Le nucléole est dense et polymorphe. Dans le cytoplasme on peut distinguer un système lacuneux composé de citernes RE de forme et dimension variables. La quantité de cytoplasme interciternale est réduite de la manière que le plus souvent les citernes se rapprochent par leurs parois. Entre les lacunes on rencontre de nombreuses mitochondries, en général de forme sphérique, ayant de cristes riches et une matrice dense. L'abondance en mitochondries prouve pour ces cellules une intense activité d'échange avec l'hémolymphe et le tissu nerveux sous-jacent.

On peut donc observer de nombreuses vésicules sphéroïdales avec un contenu homogène qui percent le système lacuneux ; elles sont présentes tellement vers la neurilème que vers la face intérieure des cellules périneuriales (fig. 1).

Le deuxième type de cellules périneuriales présente un noyau hyperchromatique avec de grands blocs de chromatine près de la membrane nucléaire (fig. 2). L'espace de la citerne périnucléaire ne garde pas une dimension uniforme comme les cellules du I^{er} type ; par endroits, l'espace s'élargit en prenant un aspect lacuneux. Cela n'empêche pas la communication de la citerne périnucléaire avec REG, et donc, ni les rapports nucléo-cytoplasmiques. Le nombre des lacunes RE est beaucoup plus réduit par rapport au premier type de cellules, mais le contenu sphérulaire homogène est présent aussi dans les cellules du deuxième type. Moins nombreuses sont les mitochondries, mais les cristes sont riches et la matrice réduite. Le cytoplasme périnucléaire contient des microtubes que l'on peut observer aussi dans le reste du cytoplasme.

La littérature nous fournit peu de données concernant l'emplacement des neurones d'origine des nerfs périsympathiqes [2]. A Gryllotalpa, ces neurones sont intra-ganglionnaires. On sait que les fibres motrices qui percent les organes neuro-hémaux périsympathiques s'en vont aux stigmes. Les fibres avec neurosécrétion, arrivées dans l'organe neuro-hémal, ont le rôle de fournir des neurohormones dans l'hémolymphe. Entre les fibres avec ou sans NS sont établis des rapports morphofonctionnels : des synapses axoaxonales, des contacts jonctionnaux étroits ou de type crampon, décrits à l'écrevisse. Dans le cas des jonctions-crampon, quelquefois dans l'espace interaxolémal se moule parfaitement un prolongement glial tellement fin que l'on peut observer seulement à un grossissement fort.

On soutient que dans le cas des jonctions électrotoniques, entre les membranes appositionnelles, reste un espace de 20—30 A, ce qui sig-

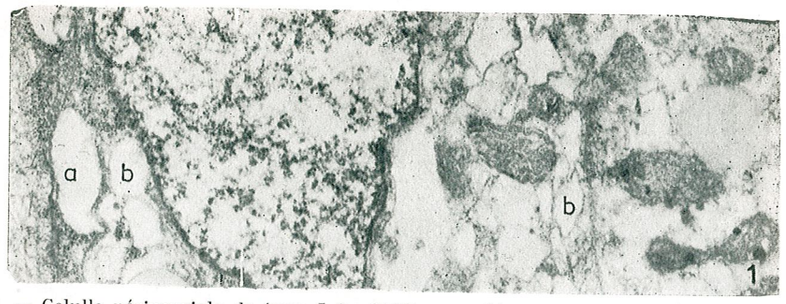

Fig. 1. — Celulle périneuriale de type I (× 3160) ; neurilème (a) ; lacunes citernales RE (b).

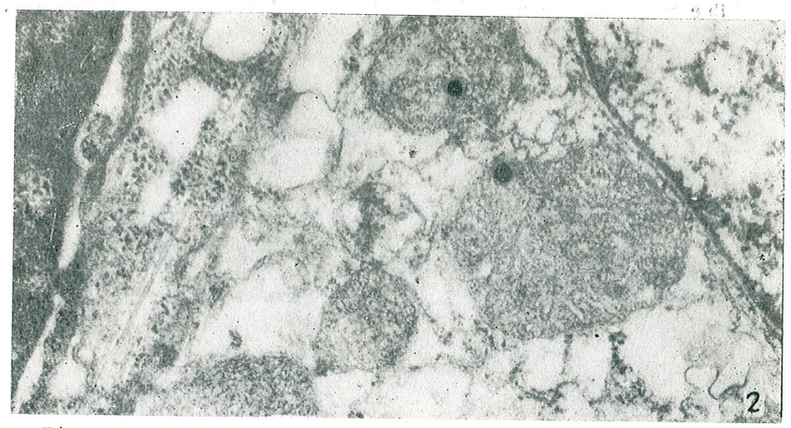

Fig. 2. — Périneurium (× 6450). Citerne périnucléaire de dimension uniforme au noyau d'une cellule du premier type (c_1) et citerne périnucléaire avec des zones élargies au noyau d'une cellule du deuxième type (c_2) ; microtubes (m) ; lacunes citernales RE (b).

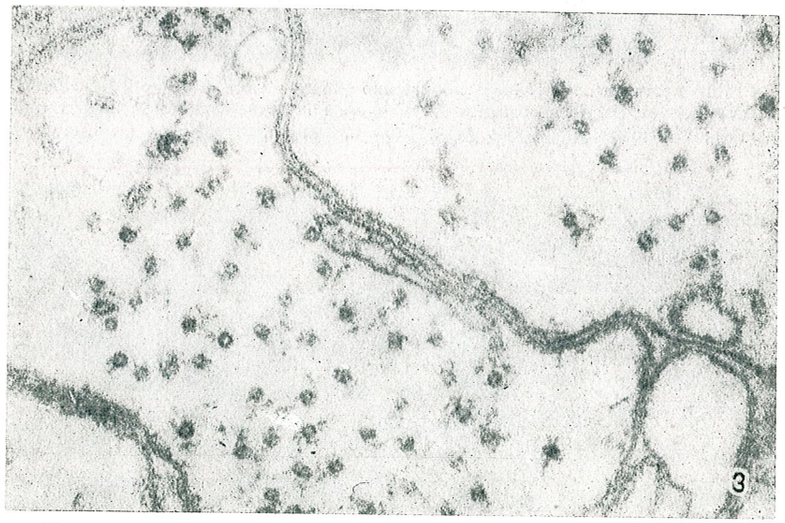

Fig. 3. — Fibres nerveuses sans neurosécrétion (× 14000). Citernes hypolémales ovoïdales (0) et applaties (t) ; neurotubes (n) ; neurofilaments (nf) ; fibres radiales (F).

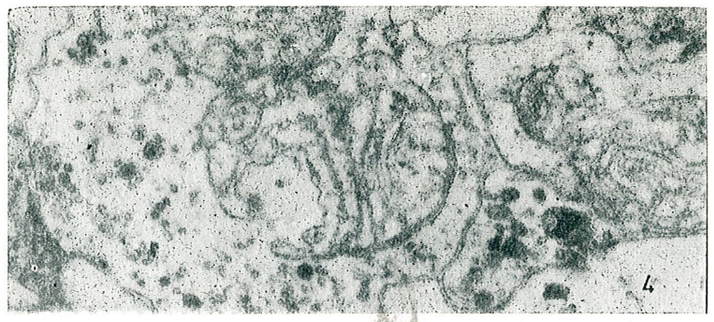

Fig. 4. — Fibre nerveuse avec neurosécrétion (× 4900). Mitochondrie déformée (M_1, M_2); cristes avec trajet ondulé (c).

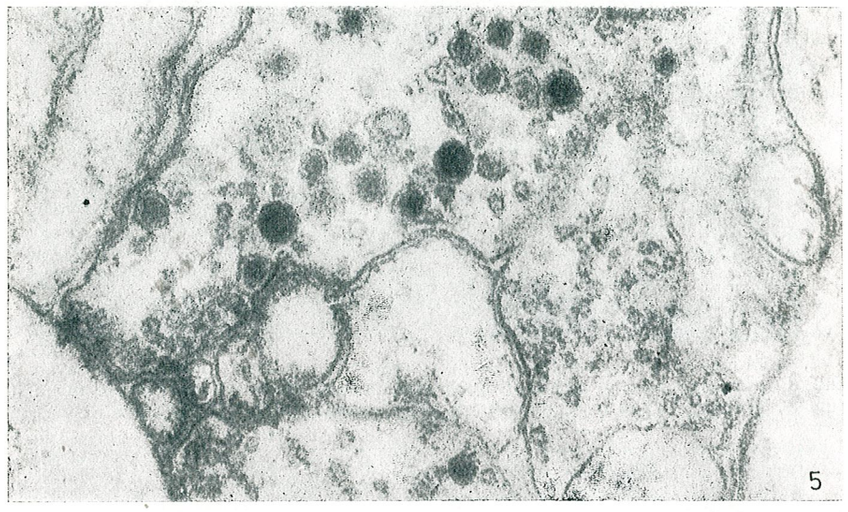

Fig. 5. — Fibre nerveuse avec NS en rapport axo-axonal et axo-glial (× 9800). Etapes d'élimination des NS par eccytose et diffusion. Etape de la formation de la membrane pentalaminaire (E); vésicules synaptiques (vs); prolongements névrogliques (a, b, c, d, e).

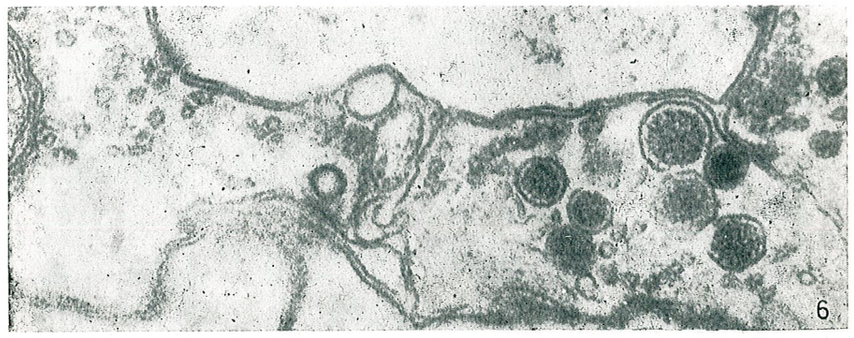

Fig. 6. — Contacts axo-axonaux (× 14000). Composante présynaptique (cp); granules NS avec un contenu dense et un contenu « structuré », ayant la membrane en partie déchirée.

nifie que les membranes ne fusionnent pas. Nous considérons que les expansions crampon solidarisent les axones en augmentant considérablement la zone de rapport interaxonal.

Les fibres sans NS contiennent dans l'axoplasme beaucoup de neurotubes équidistants placés dans la zone centrale de l'axone. Parmi eux il y a des neurofilaments solidarisés avec les neurotubes par des fibres radiales. En section transversale, les neurofilaments se montrent massifs à l'encontre de neurotubes dont le lumène est distinct (fig. 3). Dans l'axoplasme périphérique nous avons observé des éléments citernaux REN, applatis ou vésiculaires, habituellement isolés (fig. 3) mais pas amassés de la manière décrite à une autre occasion [4]. Ces citernes favorisent le transfert ionique à travers les membranes axonales. Ce transfert change ou reçoit probablement une nouvelle complexité alors qu'un prolongement glial s'interpose entre les membranes interaxonales. Peters [4] a remarqué des points adhérents entre les neurones et les prolongements gliaux. On a signalé que l'entremise des prolongements détermine l'éloignement des citernes hypolémiales. Les mythocondries intra-axonales présentent en général un aspect normal, avec des cristes tubulaires et une matrice riche; les cristes septent complètement ou en partie le compartiment intérieur. Leur trajet est rectiligne ou bien ondulé (fig. 4) Parfois les mythocondries présentent des modifications de forme, augmente le nombre des cristes en se resserant le compartiment intérieur et d'autres cristes s'élargissent et fusionnent en oblitérant ce compartiment. De telles mythocondries avec une structure altérées sont libres ou dans de vésicules autophagiques. Les corps miéloïdes plurilamellaires y sont présents.

Vers les terminaisons axonales, mais assez souvent sur le trajet des fibres, on rencontre des contacts synaptiques axo-axoniques. A l'endroit de la synapse sont amassées les vésicules synaptiques, d'habitude dans la composante présynaptique et rarement dans celle postsynaptique.

Beaucoup de fibres avec NS sont des composantes présynaptiques pour les fibres sans NS, dans le but de leur assurer probablement la trophicité et la fonctionnalité. Il faut souligner que dans les conditions de notre expérience, de tels rapports ne changent pas vis-à-vis du témoin. Les vésicules synaptiques du voisinage de la membrane synaptique sont, en général, sphériques, parfois même ellipsoïdales; quelques vésicules jonctionnent en réalisant des formations 2 ou 3 fois plus grandes que les vésicules synaptiques. Ce phénomène n'a pas été remarqué au témoin [5]. On admet que les vésicules éllipsoïdales ont un rôle inhibiteur et celles sphériques, un rôle stimulateur [3]. Les vésicules synatpiques sont groupées non seulement aux points de transmission synaptique mais aussi à l'endroit de l'émission de la neurosécrétion dans l'espace interaxonal. Cet endroit peut être situé à l'extrémité terminale des fibres NS ou sur leur trajet, ce qui suggère que les granules NS se jettent directement dans l'hémolymphe par les terminaisons axonales, mais aussi au cours du trajet axonal, directement dans les espaces interstitiels d'où elles se répandent par la neurilème dans l'hémolymphe.

Il n'est pas impossible que le transfert des neurohormones dans l'hémolymphe nécessite l'intervention des cellules gliales, surtout des cellules du premier type du périneurium, dont la richesse en mitochondries assurerait l'énergie nécessaire à un transport actif. Les granules de NS

arrivés à l'endroit de l'émission ont un contenu homogène en général, mais ayant parfois un aspect floconneux. A la périphérie, le granule présente une membrane trilaminaire, en connaissant son origine golgienne. Les granules homogènes éliminent leur contenu par exocytose habituelle : 1) la membrane du granule prend contact avec l'axolème et sur toute la zone de contact la surface du granule s'applatit ; 2) prend naissance une membrane pentalaminaire (fig. 5), puis trilaminaire et par son interruption se forme le pore ; 3) l'élimination du contenu du granule par le pore dans l'espace interstitiel.

Une autre façon d'évacuer les NS c'est la rupture partielle de la membrane du granule (fig. 6). A ces granules, le contenu dense ou « structuré » comme des flocons perd peu à peu de densité, pendant la diffusion interaxonale. Ces aspects prouvent que l'eccytose peut être supplémentée dans certains cas par le processus de diffusion de la NS.

Les images montrent précisément qu'après 48 heures de conservation *in vitro*, dans les conditions des nos expériences, le transit axonal de la NS, ainsi que son transfert dans les espaces interstitiels n'a pas cessé. L'émission de la sécrétion de la fibre nerveuse ne ressemble pas à l'eccytose habituelle d'une cellule glandulaire, même endocrine ; l'émission des neurohormones suppose la préparation de l'axolème par les médiateurs chimiques apportés par les vésicules synaptiques qui préparent de la même manière la transmission de l'impulsion nerveuse.

Nous voulons souligner ainsi notre point de vue — qui est aussi celui d'autres auteurs — concernant le double caractère fonctionnel du neurone NS, de cellule nerveuse et simultanément de cellule glandulaire.

Autour des fibres nerveuses avec ou sans NS, on rencontre des cellules névrogliques et leurs prolongements. Les contacts axone/prolongements gliaux présentent une particularité qui rapelle les rapports axogliaux du système nerveux central des vertébrés supérieurs. Ainsi, on observe dans les sections transversales, autour des axones, plusieurs prolongements gliaux ayant un aspect digité, orientés parallèlement, rarement perpendiculaires à la longueur de la fibre nerveuse. Les prolongements gliaux digités pourraient résulter de plusieures gliocytes pareillement aux vertébrés, mais ce devra être démontré.

CONCLUSIONS

Nos résultats prouvent que les organes neurohémaux situés sur le trajet des nerfs périsympathiques résistent *in vitro* pendant 48 heures dans les conditions de nos expériences. Les cellules périnucléaires développent une activité habituelle, de barrière sélective, dans le transport actif de substance vers ou bien de l'hémolymphe. Le transit axonal et l'émission de la neurosécrétion ne sont pas interrompus. Les rapports synaptiques et jonctionnaux, ainsi que ceux glioaxonaux sont aussi maintenus. Les modifications qui prouvent l'altération de certaines organites sont assez peu fréquentes pour qu'elles puissent interrompre la morphofonctionnalité des organes neuro-hémaux pendant les 48 heures de conservation *in vitro*.

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COMPARATIVE ULTRASTRUCTURAL STUDY OF PITUITARY CELLS IN FRESH-WATER TELEOSTEANS UNDER NATURAL CONDITIONS. GLYCOPROTEIC GONADOTROPHIC CELLS

BY

ZSIGMOND SZABÓ, BÉLA MOLNÁR and CONSTANTIN CRĂCIUN

The authors studied the glycoproteic gonadotrophic cells in the adenohypophysis of 8 fresh-water teleostean species in the period of sexual inactivity. In all studied species the gonadotrophic cells contain electrondense granules the mean size of which is 300 nm, as well as some slightly electrondense secretory droplets with a mean diameter of 830 nm.

The morphological identification of all pituitary cell types in lower and higher vertebrates is not yet fully clarified. Nevertheless, the morphological studies correlated with autoradiographic, immuno-electron-microscopic and other examinations have substantially contributed to the elucidation of some ultrastructural characteristics of several adenopituitary cell types [3], [4], [5]. The light and electron-microscopic studies, carried out on various teleostean pituitaries [1], [2], [4], [5], [7], [9], have shown that the proteic (acidophil) and glycoproteic (basophil) cell types exhibit seasonal modifications corresponding to the physiological state of the organism, and show specific ultrastructural characteristics as related to the higher vertebrates.

For overcoming some stress-induced ultrastructural modifications of pituitary cells during the experiments presented here, as well as for obtaining a comparative material large enough, in our experimental studies we have excluded the effect of any stress factors caused by laboratory life of animals. Furthermore, we have excluded the incertitude of the comparative study linked to the sampling of materials at various times.

In this study we present our comparative researches referring to the structural characteristics of glycoproteic gonadotrophic cells in 8 fresh-water teleosteans (Cyprinidae). All species were studied in the period of sexual inactivity.

MATERIALS AND METHODS

Sexually mature individuals of both sexes were collected from the Someșu Mic river (at Gilău), in September 1976, using an electric aggregate apparatus. In a total number, there were collected 54 pituitaries for

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light- and electron-microscopic studies immediately after fishing the animals, i.e. in their electro-shock state. The pituitaries were isolated from the following species : *Barbus meridionalis petényi* (9 individuals), *Gobio gobio carpathicus* (8 individuals), *Alburnoides bipunctatus* (6 individuals), *Leuciscus cephalus* (5 individuals), *Phoxinus phoxinus* (6 individuals), *Chondrostoma nasus* (4 individuals), *Nemachilus barbatulus* (8 individuals), *Cobitis taenia* (8 individuals). All species were found in the post-reproduction period, when in their ovaries the vitellogenesis was found to be intense. For light-microscopic purpose, the pituitaries were fixed in *Bouin* solution and embedded in paraffin. The serial sections of 5—7 μm were stained with AZAN. In the electron-microscopic study the material was fixed for 90 minutes in 2.5% glutaraldehyde and after that fixed in 1% osmium tetroxide solution. For embedding the materials, vestopal W has been used. The sections contrasted with uranyl acetate and lead citrate, were examined by using an electron microscope of TESLA BS-613 type.

RESULTS

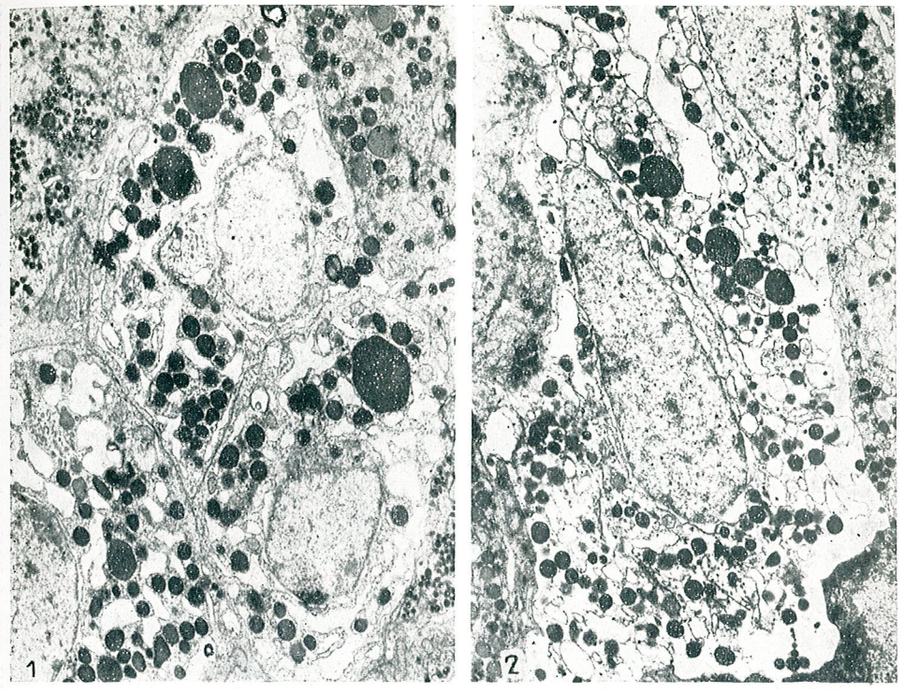
The great majority of gonadotrophic cells in all species studied here are present in the mezoadenohypophysis, and in a reduced number in the proadenohypophysis. The size of these cells is relatively large (about 20 μm in diameter) and, generally, they have abundantly vacuolized cytoplasm. These cells having more electrondense granules in their cytoplasm than other cell types, as well as because they contain less electronodense droplets (globules), may easily be recognized by electron-microscopy.

Table 1

No.	Species	Diameter in nm of : granules mean	granules variation	droplets mean	droplets variation
1	*Barbus meridionalis petényi*	310	175—450	1000	670—1200
2	*Gobio gobio carpathicus*	300	160—470	—	—
3	*Alburnoides bipunctatus*	300	175—470	700	580—760
4	*Leuciscus cephalus*	300	175—470	950	870—1050
5	*Phoxinus phoxinus*	300	220—400	600	500—700
6	*Chondrostoma nasus*	310	170—450	650	500—700
7	*Nemachilus barbatulus*	310	230—410	920	700—1400
8	*Cobitis taenia*	310	210—470	1040	980—1100

In all species studied here, the size of both granules and droplets is obviously uniform (see Table 1). The mean size of granules is 300 nm, while that of droplets is 830 nm (Figs. 1 and 2).

Another ultrastructural characteristic of these cells is the presence of dilated endoplasmic reticulum cisternae, which give a vacuolar aspect to the cytoplasm. The rough endoplasmic reticulum (RER) has a diffuse aspect, which is more evident around the nucleus. In the great majority



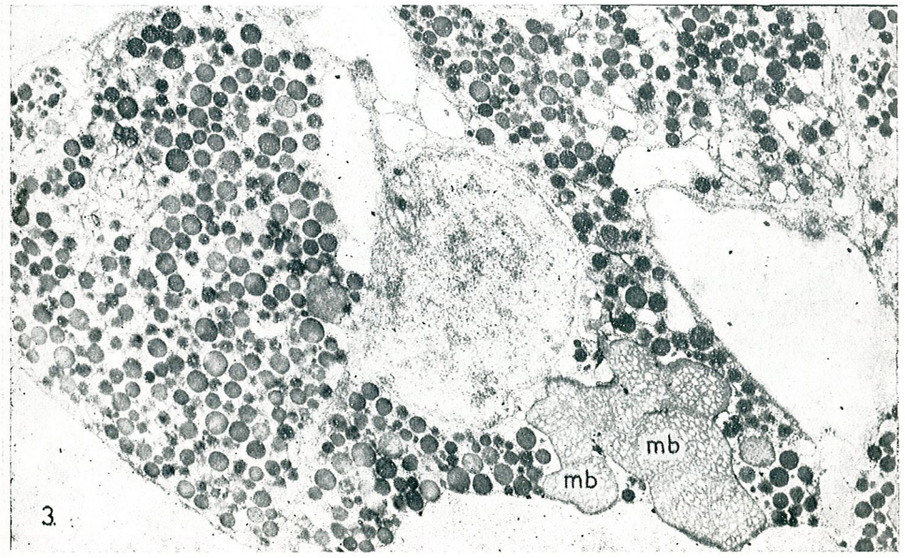
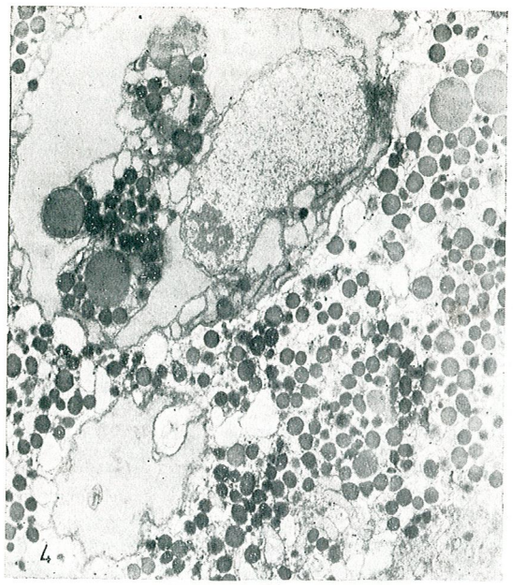
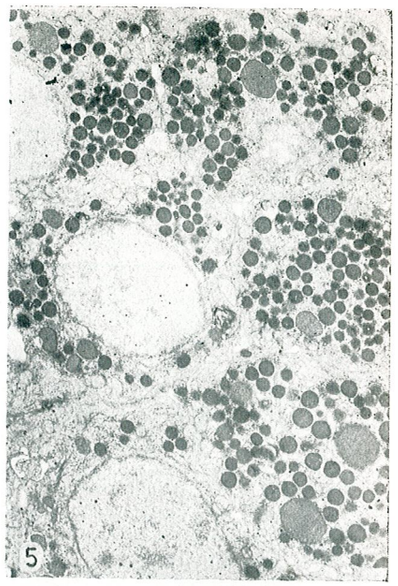


Fig. 1. — Glycoproteic gonadotrophic cells in mezoadenohypophysis of *Nemachilus barbatulus*. × = 7,500.

Fig. 2. — Two intense vacuolized gonadotrophic cells in mezoadenohypophysis of *Phoxinus phoxinus*. × = 7,500.

Fig. 3. — Irregular aggregate of multivesicular bodies (mb) in a glycoproteic cell of *Gobio gobio carpathicus*. × = 8,000.





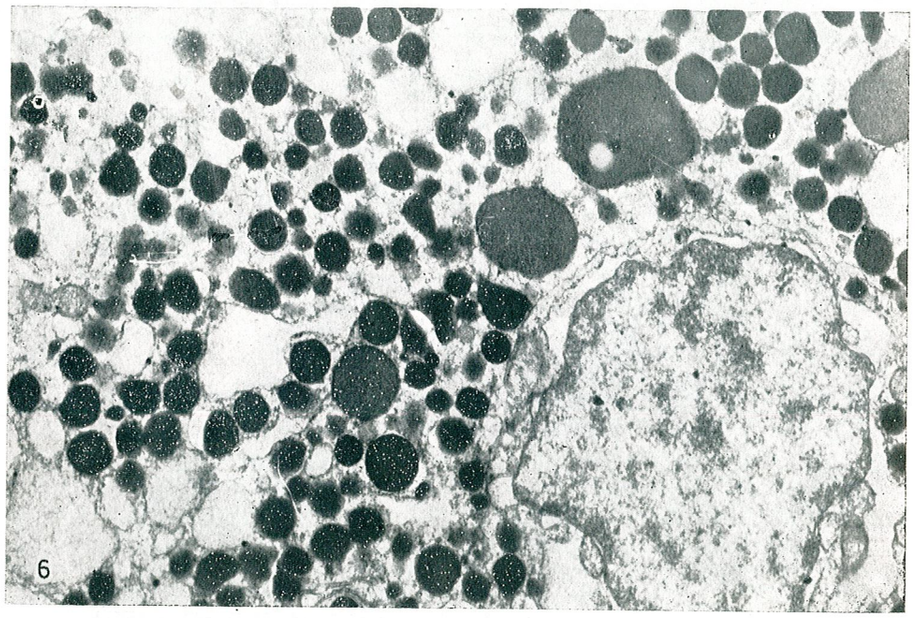


Fig. 4. — Extremely dilated RER cisternae in a gonadotrophic cell from the mezoadenohypophysis of *Leuciscus cephalus*. × = 7,300.

Fig. 5. — Slightly vacuolized gonadotrophic cells in mezoadenohypophysis of *Cobitis taenia*. × = 6,500.

Fig. 6. — Intensely vacuolized gonadotrophic cells in mezoadenohypophysis of *Chondrostoma nasus*. × = 18,000.

of cases, the constituents of Golgi complex are slightly evident and present in the perinuclear zone. In *Phoxinus* and *Nemachilus* we have also observed the presence of some smaller cells with darker cytoplasm, without dilated RER cisternae.

Generally, the mitochondria are present in a great number and have an elongated aspect. In a great number of species we have established the presence of some gigantic irregular corpuscles of about 3 μm in diameter, each being constituted by aggregation of many multivesicular bodies of 0.5—0.8 μm in diameter (Fig. 3).

The spheric or ovoid nucleus relatively reduced is present in two forms : i.e. a normal morpho-functional aspect, and a pycnotic aspect, with an irregular outline and deep infoldings.

Almost in all studied species it may be observed the presence of large vacuoles with a low electrondense content. In the *Leuciscus cephalus* (Fig. 4) we have found exaggerated dilatation of RER with a uniform and moderate electrondense content in their cisternae.

DISCUSSIONS

In our researches we started from the idea that in teleosteans-similarly as in batrachians — the gonadotrophic hormone producing cells (FSH and LH) are mainly located in mezoadenohypophysis and containing glycoproteic granules, they present the following cytological characteristics : are relatively large and basophil, their secretory granules are small and stained with anilineblue, being PAS and AF positive ; the secretory globules (droplets) are large (600—1400 nm) can be stained with orange-G and erythrosine and give PAS and AF positive reactions. The cytoplasm of these cells presents an obvious vacuolization in the majority of cases.

At the moment of examination both the male and female individuals of 8 teleostean species were found in an inactive stage of their sexual cycle. Knowing in general the yearly seasonal cycle of these species [9], we may affirm that during this season the vitellogenesis is intense in the ovaries. This affirmation seems to be effective for both species having synchronic ovogenesis and a single egg-laying per year *(Chondrostoma, Leuciscus, Alburnoides)* and the species having asynchronic ovogenesis with more eggs-laying per year *(Barbus, Gobio,* etc.).

Comparing the data obtained with light microscopy to those obtained with electron microscopy, it is pertinent to conclude that in the ultrastructure of gonadotrophic glycoproteic cells there are reflected both the active and inactive phases of secretion. The electron-microscopic aspect of a great number of gonadotrophic cells show the phenomenon of a gradually absorption of non-released secretory granules.

It may be assumed that the electrondense secretory granules being not released, after a time they are linked to the primary lysosomes and by fusion it is formed an aggregate of multivesicular bodies which have 3 μm in diameter. Furthemore, it may be assumed that after a total absorption of their content, these multivesicular bodies are transformed

in vacuoles. The vacuolization leads to an almost total disaggregation of the cytoplasm, without affecting the integrity of the nucleus.

Besides the cells in which an intense vacuolization takes place, in the pituitary there are also present some nonvacuolized glycoproteic cells having a dark hyaloplasm and intense electrondense secretory granules (Fig. 5). It is to underline the fact that we have observed an obvious and frequent vacuolization in those species which have a synchronous vitellogenesis *(Alburnoides, Leuciscus, Chondrostoma)* (Figs. 4 and 6).

CONCLUSIONS

From the comparative ultrastructural study of glycoproteic gonadotrophic cells in 8 cyprinidae species (collected from natural environment, from the same biotope and at the same time) results that these cells contain secretory granules and droplets (globules). In all studied species the intense electrondense secretory granules have a mean size of 300 nm, while the mean diameter of slight electrondense droplets is of 830 nm. Some cells with dark cytoplasm present signs of elaborating activity of the secretory product, others are vacuolized and show a pronounced diminution of the cytoplasm without affecting the cell nucleus integrity. This cytologic aspect is characteristic of the inactive sexual period in September.

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ELECTROFEROGRAMAS DE LAS PROTEINAS MUSCULARES DE CUATRO ESPECIES DE SCIAENIDAE (PISCES) DE AGUAS CUBANAS

POR

TSAI GARCIA

Proteins electrophoresis of the white muscle of four species of Sciaenidae *(Micropogonias furnieri, Bairdiella ronchus, Equetus lanceolatus* and *Pareques acuminatus)* was performed. Each species showed one specific pattern, independently from the sex and size of the specimens. According to the pattern of muscular proteins, a closer phylogenetic relationship may exist between *P. acuminatus* and *E. lanceolatus*, while the *B. ronchus* electrophoregram shows the biggest differences as against the rest of the species.

Estudios taxonómicos aplicando la técnica de la electroforesis se han venido realizando por diversos autores [3], [4]—[7]., los cuales han probado que las proteinas del músculo presentan un patrón específico para cada especie. Así mismo, el modelo de las proteinas musculares tiene valor para analizar las relaciones filogenéticas entre los diferentes grupos bajo una familia [9].

Las relaciones filéticas entre los géneros de Sciaenidae del Atlántico oeste fueron propuestas por Chao [1] basándose en la estructura de la vejiga de los gases, otolitos y morfología externa. En el presente trabajo se establecen las afinidades entre 4 especies de esciaénidos pertenecientes a 4 géneros diferentes, de acuerdo a un criterio bioquímico.

MATERIALES Y MÉTODOS

Se tomaron muestras de 20 ejemplares de *Micropogonias furnieri* y *Bairdiella ronchus* procedentes de la Bahía de Cienfuegos y 13 ejemplares de *Equetus lanceolatus* y *Pareques acuminatus* colectados en el litoral de La Habana.

Los peces fueron congelados inmediatamente después de su captura. Se tomó 1 g de músculo blanco de la región dorsal del pez y se homogeneizó con 4 ml de agua destilada. El homogenado se centrifugó durante 20 minutos a 4000 rev/min. a temperatura ambiente. El sobrenadante obtenido fue usado para la electroforesis. La separación de las proteinas se realizó en gel de poliacrilamida según el método de Chapel et al. [2]. La electroforesis se realizó con una corriente de 9 mA por cm durante 2 horas y 30 minutos. El gel fue tenido con negro amido y decolorado con una solución de ácido acético al 7.5%.

RESULTADOS

DESCRIPCIÓN DE LOS ELECTROFEROGRAMAS DE LAS DIFERENTES ESPECIES

En el modelo electroforético de *Bairdiella ronchus* se obtuvieron 6 bandas con diferentes intensidades, grosor y velocidad de migración. En la Fig. 1 A se observan las bandas 1, 2 y 3 con una migración semilenta, las bandas 4 y 5 con migración intermedia y la última fracción (6) con una velocidad de migración rápida.

El electroferograma de *Micropogonias furnieri* mostró 6 bandas donde predominan las fracciones de desplazamiento semilento (2—4).

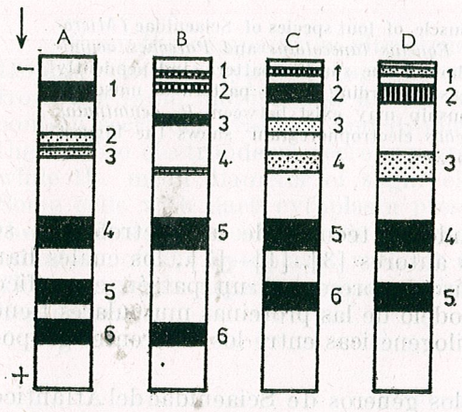

Fig. 1. — Electroferogramas de *Bairdiella ronchus* (A); *Micropogonias furnieri* (B); *Pareques acuminatus* (C); *Equetus lanceolatus* (D).

La fracción 5 tiene una velocidad de migración intermedia y la 6 es de migración rápida (Fig. 1 B).

Pareques acuminatus tiene un patrón electroforético constituido por 6 bandas (Fig. 1 C). La banda 1 tiene una migración lenta, mientras que las fracciones 2—4 se desplazan semilentamente y las bandas 5 y 6 presentan una velocidad de migración intermedia.

Equetus lanceolatus presenta 5 bandas (Fig. 1 D) estando ausentes proteinas con velocidad de migración rápida.

COMPARACIÓN ENTRE LOS ELECTROFEROGRAMAS DE LAS 4 ESPECIES

Cada especie de Sciaenidae mostró un patrón específico al realizarse la electroforesis de las proteinas del músculo, independiente del sexo y del tamaño de los ejemplares.

En las 4 especies estudiadas fue común la presencia de una banda (la número 4 para *B. ronchus* y *E. lanceolatus* y la 5 para *M. furnieri* y *P. acuminatus)* la cual tuvo la misma velocidad de migración, intensidad y grosor. En todas las especies predominan las proteinas con migración semilenta e intermedia.

Tsuyuki et al. [10] determinaron que la posición de las zonas era un criterio para las relaciones entre las especies y reconocieron que a mayores afinidades entre les especies, más número de zonas tenían en común.

De acuerdo al patrón de las proteinas del músculo, parece existir la mayor relación filogenética entre *P. acuminatus* y *E. lanceolatus;* así como, el electroferograma de *B. ronchus* muestra las mayores diferencias con el resto de las especies.

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EFFECTS OF CROWDING ON THE HISTOLOGY AND HISTOCHEMISTRY OF THE ADRENAL CORTEX IN MALE ALBINO MICE

BY

ANCA PETRESCU-RAIANU, T. TRANDABURU, VALERIA POPESCU

Subjecting male mice to crowding for 2, 4, 24 or 48 hours histological changes, such as a marked enlargement of capillaries, adrenal hemorrhage, cellular hypertrophy and increased frequency of the dark cells were noted. They are associated with decreased amounts of lipids, free and esterified cholesterol and ascorbic acid. Crowding appears to be a stressful stimulus that enhances adrenocortical function. The deaths recorded among animals housed in highly crowded groups suggest that the psychosocial stress might be a mechanism for population limitation.

Cyclic density changes of rodent natural populations is at present a well-known phenomenon. As far as 1950, Christian has explained the abrupt decline occurring after a population reached a high level of density, by neuroendocrine disorders, patricularly by functional disturbances of the pituitary-adrenal and pituitary-gonadal axes [10]. The stress capacity of high density levels was confirmed by numerous studies carried out on natural populations as well as on groups assembled in laboratory for short periods. It was established that the neuroendocrine disorders are induced by increased incidence of social contact between the individuals of a group. Under crowded conditions increased aggressiveness [2], [7], [17], [20], abnormal behaviour of subordinates [4], [9], [22], and decreased reproduction ability [5], [16], [19], [21], [24], [25], were described. The subordinates showed loss of the brain [4], and thymus weights [11], lessions of renal glomeruli [2], [22], hepatic necrosis [2].

There is an extensive literature concerning the direct relationship between adrenocortical function and social interaction in the rodent populations. The subordinate members of the crowded populations show an increase in adrenal weight [1], [3], [16], [20], enhanced adrenocortical activity [1], [2], [5], [6], [21], associated with an elevation of plasma corticoid levels [12], [26]. Although there are many studies revealing a psychosocial stress-induced increase in the adrenal weight, the histological and histochemical changes of adrenal cortex are not well-known. Therefore, we thought to be of interest to study the effects of short periods of crowding upon adrenal cortex structure.

MATERIAL AND METHODS

Experimental mice were housed in pairs (a male and a female) from weaning until sexual maturity. Food and water were provided *ad libitum*. After the sexual maturity was reached, the males were placed in larger

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pots (28 cm in diameter) in groups of fifteen or twelve. They were killed by decapitation after 2, 4, 24, and 48 hours of crowding. Ten males that remained in pairs with their females were used as controls. The killing was always carried out at the same time of the day.

The adrenals were dissected out and processed as follows :

— for histological study they were fixed in Bouin's fluid and paraffin sections were stained with hemalum and eosin, or Mallory's trichrome ;

— for total lipids demonstration, the adrenals were fixed in Baker's formol-calcium and the sections obtained with a cryotom were stained with Sudan black B ;

— for free and esterified cholesterol demonstration, the formol-calcium fixed material was cut in the cryotom, digitonin treated, with or without Sudan black B stain and examined in polarized light ;

— for ascorbic acid demonstration, the method of reducing silver nitrate after Sosa procedure was used [15]. The relative quantitative estimation was performed by reduced silver granules counting per area unit by means of an eye-piece with a network. The measurements were carried out in the three zones of the cortex. The statistical significance of ascorbic acid quantitative differences was determined by Student's t test.

RESULTS

Immediately after the mice grouping under crowding conditions, a remarkable excitation state occurred, accompanied by violent fighting among all members of the group. The first fight lasted between ten and fifteen minutes, depending on the group, and it was followed by a short, quiet period. In the first four to six hours of the experiment the fights were repeated for short periods of five to ten minutes, which were alternated with quiet periods. One or two individuals in each group of fifteen died in the first two to three hours from the beginning of the experiment, while there was no death in the groups of twelve. Other two to three individuals showed a serious exhaustion state. Most animals had visible wounds. The individuals which died were replaced by others, reared under the same housing conditions and marked to be distinguished from those initially ones, used in the experiment.

The histological structure of the adrenal cortex in controls was identical with that described for this species in the literature (Fig. 2), [13], [14], [23]. After two hours of crowding a marked enlargement of capillaries between the adrenal cords was noted throughout the cortex. This feature was found in the animals sacrificed after four hours of crowding, too, but it was uncommon after 24 or 48 hours. In these last ones a marked enlargement of capillaries occurred only in the inner zones and at the cortico-medullary border (Fig. 4). This process was more important in some individuals, leading to adrenal hemorrhage (Fig. 5).

Subjecting the mice to crowding for four hours, numerous dark cells with basophilic nuclei were noted in the inner zones of the cortex

(reticular and inner part of fascicular zones). The dark cells were regularly found in the adrenal cortex of individuals maintained 24 hours under crowded conditions. They occurred in noticeable areas that reached the outer fasciculata (Fig. 3).

The hypertrophy of adrenocortical cells was obvious after four hours of crowding. The individuals maintained for longer periods under these conditions showed numerous hypertrophic nuclei not only in the inner zones, but also in the outer part of fasciculata (Fig. 7). Some of these nuclei contained vacuoles (Fig. 6). In addition, the reticular zone of these individuals exhibited many cells with strong eosinophilic cytoplasm and pycnotic nuclei.

The amount of sudanophilic lipids changed depending on the period length of animal maintaining under crowding conditions. In the fascicular zone of the controls, it was larger than that in the reticular one (Fig. 8). After two hours from the beginning of the experiment, the glomerular and outer fascicular zones lost, in part, their lipids (Fig. 9). The amount of lipids in the fascicular zone continually decreased during 48 hours, while in the reticular zone after 48 hours they were found to be increased.

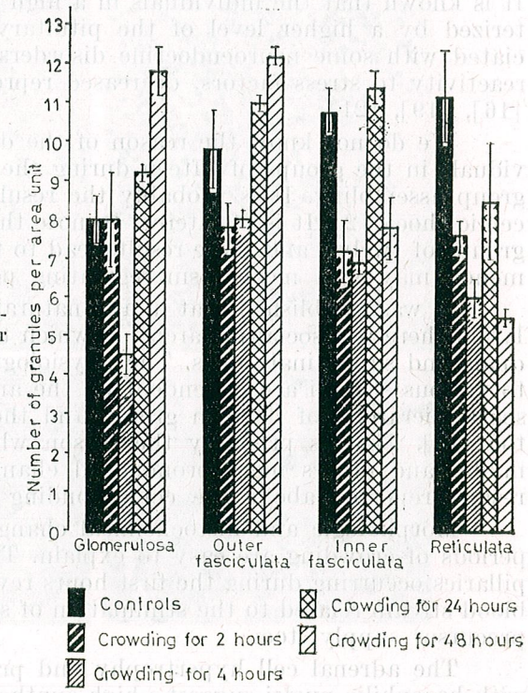


Fig. 1. — The ascorbic acid quantitative changes in adrenal zones after various periods of crowding.

The esterified cholesterol was preponderant both in experimental mice and controls. The crowding caused a gradual decrease in both cholesterol fractions, proportional to the duration of animal maintaining under experimental conditions. 48 hours after animal grouping, marked

depletion in adrenal free cholesterol was noted, while the cholesterol esters were found only in the fascicular zone, in a very small amount (Fig. 15). It was noticed that fasciculata was the only place where cholesterol was found alfter 48 hours of crowding.

The ascorbic acid quantitative changes among adrenocortical zones after various intervals of crowding are summarized in Fig. 1. The most important and rapid changes occurred in the inner fascicular and reticular zones, where the lowest levels were found after 4 hours. After 24 hours from the beginning of the experiment there was an increased amount of ascorbic acid, similar to that of controls, but it significantly decreased again in the inner zones after 48 hours.

DISCUSSION

The irritability and violent fighting occurring immediately after the mice grouping are in agreement with literature data concerning the increase in aggressivenes in high density populations [2], [18], [20]. It is knowh that the individuals in a high density population are characterized by a higher level of the pituitary-adrenocortical function associated with some neuroendocrine disorders (abnormal behaviour, altered reactivity to stress factors, decreased reproduction ability) [2], [7], [8], [16], [19], [21].

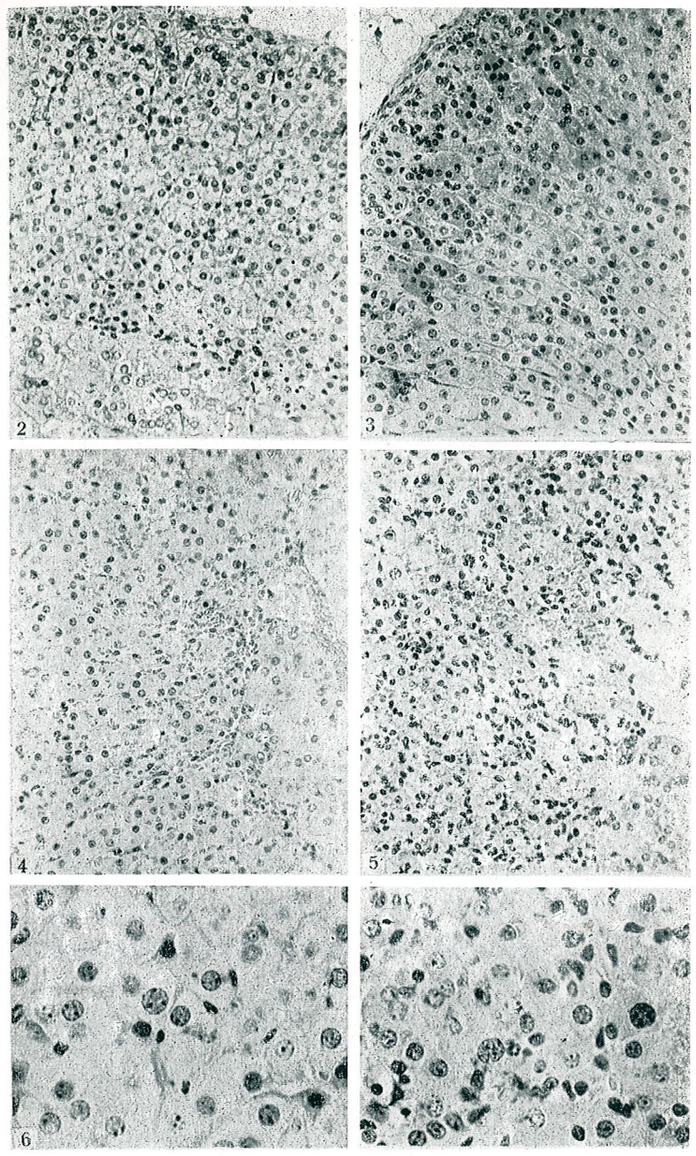
We do not know the reason of the death of the two or three individuals in the groups of fifteen during the first two to three hours after group assembling. It is probably the result of hypertension or hypoglycemic shock [2]. It is of interest to note that there were no deaths in the groups of twelve, and these results lead to the conclusion that this phenomenon may be a mechanism regulating population density.

It was established that in any natural or experimental rodent population there is a social hierarchy in which there are higher ranking individuals and subordinate ones. The physiological and behavioural responses to various stimuli are dependent on the animal's relative position in the social hierarchy of its own group, and the nature of social interaction, too [18]. That is probably the reason why some individuals displayed milder and others more pronounced changes that the mean ones. Our results reported above are corresponding to most animals' status.

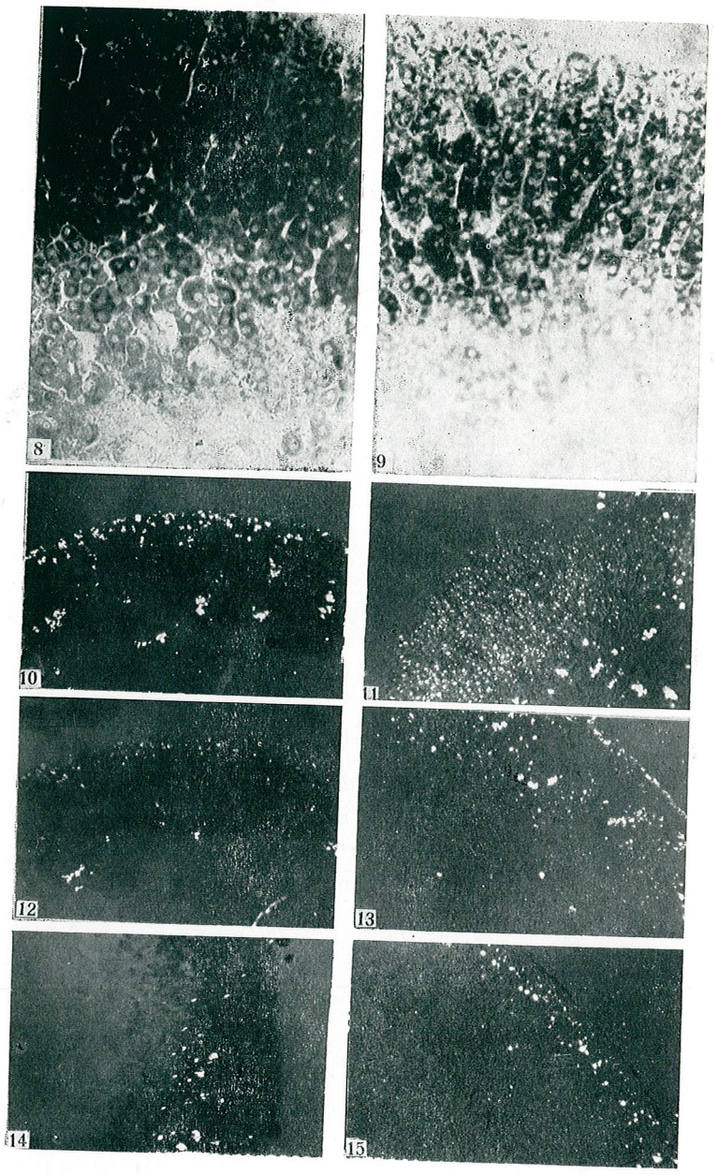
Morphologic and histochemical changes observed by us after short periods of crowding are easy to explain. The marked enlargement of capillaries occurring during the first hours reveals an increase in the adrenal blood stream related to the stimulation of steroid output, and an elevated precursor supply, too.

The adrenal cell hypertrophy and preponderance of the dark cells with basophilic nuclei suggest a high synthesis of steroid hormones. Reduced amounts of sudanophilic lipids, cholesterol and ascorbic acid lead to the same conclusion.

Unlike outer fasciculata, the deeper cortical zones (inner fasciculata and reticulata) were the sites where the most important and rapid changes



Figs.2—7.— 2, Section of the adrenal gland from a control mouse ; 3, Section of the adrenal gland from a mouse sacrificed after 24 hours of crowding. Note the hypertrophy of the adrenocortical cells and the presence of dark cells in fasciculata ; 4, Section of an adrenal cortex showing a marked enlargement of capillaries after 4 hours of crowding ; 5, Adrenal hemorrhage in the cortical inner zones of a mouse maintained 24 hours under crowded conditions ; 6, Hypertrophic nucleus with a large vacuole in the inner portion of the fascicular zone from a mouse sacrificed after 24 hours of crowding ; 7, Two hypertrophic nuclei and several pycnotic ones in the reticular zone from mice maintained 48 hours under crowded conditions ;



Figs.8—15.—8, Section of the adrenal gland from a control mouse histochemically stained with Sudan black B for demonstration of lipids; 9, Section of the adrenal gland from a mouse sacrificed after 2 hours of crowding. Note the decrease in the lipid content of cortex; 10, Free cholesterol in the adrenal cortex of a control mouse; 11, Total cholesterol in the adrenal cortex of a control mouse; 12, Free cholesterol in the adrenal cortex of a mouse sacrificed after 4 hours of crowding; 13, Total cholesterol in the adrenal cortex of a mouse sacrificed after 4 hours of crowding; 14, Free cholesterol in the adrenal cortex of a mouse maintained 24 hours under crowded conditions; 15, Total cholesterol in the adrenal cortex of a mouse maintained 48 hours under crowded conditions.

occurred, revealing that the first one may play some role in storage of precursors or other substances taking part in biosynthesis of corticosteroid hormones.

Quantitative changes of lipids, cholesterol and ascorbic acid become visible within the groups of mice killed two hours after the beginning of the experiment, but they were more marked in adrenals of the mice sacrificed after longer periods. The amounts of lipids and ascorbic acid returned more or less near to the initial level after 48 and 24 hours respectively. This fact might be interpreted as an attempt of the organism to adapt to the new conditions by mobilizing of the adrenal functional potentialities. This idea is also supported by other findings; for example it was established that after a time the pituitary-adrenal activity could return to a pre-stress level even when the animals continued to be exposed to the stressful stimulus, so that they are presumed to be normal. But this status is only an adaptation, and it was shown not to be synonymous with a normalization, because they are more responsive to a second stress stimulus [27].

On the basis of the above results one might conclude that the crowding is a stressful stimulus which may be due to a greater frequency of social interactions. Under crowded conditions, until the social hierarchy is established, violent fighting occurred, and that is reflected in adrenal morpho-functional status; in crowded mice there are obvious adrenal structural changes corresponding to enhanced corticosteroid synthesis. Attention must also be given to the possibility that psychosocial stress might be a population-regulating mechanism in rodents, suggested by the deaths in animals housed in groups of fifteen in contrast to animals at lower population density.

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HISTOLOGICAL CHANGES OF THYMUS IN HYPERINSULINISM AND ALLOXANIC DIABETES

BY

MARIA-SENA CRIVII, G. SIMU and E. A. PORA

Histological alterations of rat thymus after acute and chronic treatments with insulin as well in alloxanic diabetes were investigated in white Wistar rats. Following treatment with high doses of insulin and alloxanic diabetes, both animals showed a significant thymic involution and an increase of the number of mast cells containing alcian blue stained granules. This was interpreted as a consequence of adrenals stimulation. All modifications depended on the dose of insulin administered and disappeared after bilateral adrenalectomy.

INTRODUCTION

The influence of insulin on the thymus is an older problem of endocrinology [2], [12], [21]. In the last decade, the relation between pancreas and thymus was raised again [3], [13]. According to Zeckwer, great, repeated doses of insulin induce a thymus and lymph node involution associated with adrenal medulla hyperplasia. This author thinks that insulin rises the adrenalin secretion of the gland, activating by this mechanism the corticoadrenals and potentiating antilymphatic effect [1], [6], [9], [10], [18].

In this series, the present paper follows the histological patterns in rat thymus subsequent to an insulin acute and chronic treatment, as well as in animals with alloxanic diabetes.

MATERIAL AND METHODS

The experiments were performed on Wistar male rats weighing 90 ± 10 g maintained in standard conditions. CIF insulin was injected intraperitoneally à jeun. The insulinic shock was induced by doses of 2 IU/100 g body weight [21]. For chronic treatment (3 weeks) the following groups were used :

1. Controls (saline buffered at pH 2–3 with glacial acetic acid).
2. 0.1 IU/day
3. 0.25 IU/day
4. 0.5 IU/day
5. 1.0 IU/day (in 2 doses)

The alloxanic diabetes was obtained in normal and adrenalectomized animals after the original method of the Animal Physiology Institute of Cluj-Napoca [11]. After the measurement of glycemia, the animals were beheaded and the thymus was collected and studied histologically in preparations stained with hematoxylin-eosin and alcian blue-safranin for mast cells [4].

RESULTS AND DISCUSSIONS

Table 1

Mean of number of thymic mast cells with alcian blue stained granules, standard error (± ES), *t* and *p*, of insulin and alloxanized Wistar rats

Values	Control	Treated with insulin			Adrenalectomy and insulin shock	Alloxanic diabetes	Adrenalectomy and alloxanic diabetes
		shock	chronic				
			0.25 UI	1 UI			
n	5	7	7	7	7	8	8
X	6.8	27	20.42	19.42	6.42	19.62	6.75
ES (*t*)	0.37	1.09	1.06	1.02	0.36	0.65	0.36
t	—	15.07	15.52	10.06	0.71	14.56	0.9
p	—	<0.001	<0.001	<0.001	>0.5	<0.001	>0.5

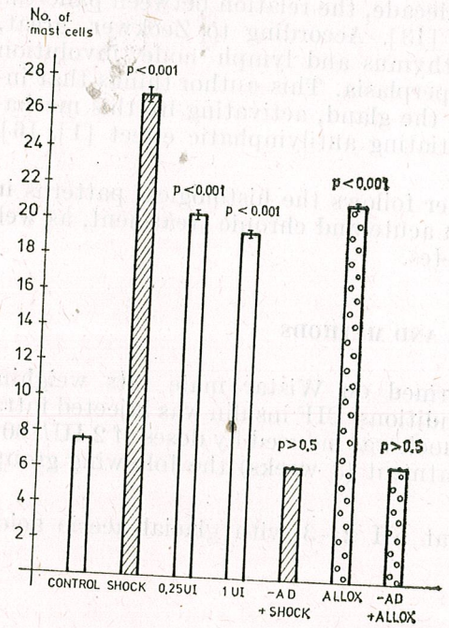

Fig. 1. — Number of thymic mast cells with alcian blue stained granules of control and treated animals (UI = international units, AD = adrenalectomy; allox = alloxanic diabetes).

The results are presented in table 1 and figure 1. Each individual value represented the mean of three microscopically fields (ob. 25).

In controls, rare mast cells, especially containing safraninophil granules, exceptionally alcianophil, were observed. ($X = 6.8$).

In animals with insulinic shock, besides a lymphocyte depletion leading to the disappearance of the difference between medulla and cortex and a hyperplasia and hypertrophy of the reticuloepithelial cells generating Hassall's corpuscles, a frank multiplication of the mast cells containing predominantly or solely blue granules was seen ($\bar{X} = 27$; $t = 15.07$, $p < 0.001$).

The bilateral adrenalectomy blurs these changes, the animals exhibiting a large thymus with rich lymphocytary cortex and only rare blue mast cells. ($\bar{X} = 6.42$ $t = 0.5$ $p > 0.5$)

In animals chronically treated with insulin, no changes in comparison with controls were found for a dose of 0.1 IU/day. In contrast, doses of 0.25, 0.5 and 0.75 IU/day increased the number of mast cells, especially of those containing blue granules. Mast cell behaviour in animals treated with 1 IU, an undershocking dosis, is very similar to that seen in animals treated wiht 2IU, their number is increased, cells with blue granules being predominant ($\bar{X} = 19.42$, $t = 10.06$ $p < 0.001$).

In animals with alloxanic diabetes, a diminished thymus with a starry sky pattern in the thinned cortex was seen. No frank cellular rhexis was observed, although the presence of granular intercellular material may suggest such a phenomenon. An acidophil dystrophy of reticuloepithelial cells evolving to Hassall's corpuscles was seen in medulla. The difference between cortex and medulla disappeared in several areas. The subcapsullar mast cells were multiplied, besides the predominating cells with blue granules existing numerous cells with safraninophil ones. $X = 19.62, t = 14.6$ $p < 0.001$).

In adrenalectomized and subsequently alloxanized rats, the thymus increased in volume, with compact lymphocytary cortex. Only rare blue granules containing mast cells were seen subcapsulary; most cells were in course of degranulation ($\bar{X} = 67.75$, $t = 0.9$; $p > 0.5$).

These results are discussed from the unanimously accepted point of view of stimulating corticoids secretion by insulin and of their suppression by bilateral adrenalectomy [22]. The relation between thymus and adrenals is well known [1], [3], [6]—[10], [17]. During stress or subsequent to a treatment with cortisone, the lymphocyte lysis in the thymus is accompanied by an increased mast cells formation [17]. It seems that, at least in rat and mouse, the thymus is the preferential organ of the mast cells formation, the inductor of this process being cortison [6]—[8]. As concerning the mast cells behaviour during the acute and chronic treatment with insulin, our results are coincident with those of Csaba *et al.* [5]. However, the question is raising how the increase of the mast cell number is possible during the short time of 30—40 minutes in which the insulinic shock becomes obvious [5]. The experiments of Csaba *et al.* using prednison showed the presence of mast cells in the thymus capsule and adjacent tissues, whose number was much increased after 3 days of treatment.

The changes concerning Hassall's corpuscles in diabetic animals are similar with those described in the literature [15], [19]. The increased number of these structures expresses an accidental involution of the thymus. The lack of insulin may constitute a stress producing a thymus involution more expressed in younger animals whose organ is not yet markedly involuted.

The fact that Wira and Munck [20] as well as Munck [13] identified in thymus cell nuclei specific receptors for cortisol argues for the hypothesis that diabetes influences the thymus also by the hypothalamus — hypophysis — corticoadrenal channel. In animals with alloxanic diabetes, the bilateral adrenalectomy blurs these changes, and the mast cell number and the chromophilia of these cells are similar to those of the controls.

CONCLUSIONS

1. The effects of insulin administration on the thymus are dependent on the dosis and the route of administration.

2. Thymus involution under the action of insulin and in alloxanic diabetes is the consequence of adrenals stimulation.

3. The number of mast cells containing alcian blue stained granules shows the quantity of corticoids secreted acutely or chronically and expresses the degree of thymus involution.

4. These modifications vanished after bilateral adrenalectomy.

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PROCAINE EFFECT ON MEMBRANE POTENTIAL IN HIGHCALCIUM MEDIUM

BY

I. NEACȘU and ȘT. AGRIGOROAEI

The effect of procaine on membrane potential of frog sartorius muscle fibers in Ringer solution with 5 mM Ca^{2+} at pH = 7.2 and 6.0, using glass intracellular microelectrode method has been investigated. The increase of external Ca^{2+} concentration brings about a hyperpolarization of the membrane with higher amplitude in acid than in alkaline conditions. This phenomenon is based on a phase transition from globular phospholipidic micellae of the membrane external layer to laminar micellae. The procaine antagonized totally the Ca^{2+} effect at pH=7.2 and partially at pH = 6.0. At low procaine concentrations (1 mM) the effects of the two agents are additive.

The investigations regarding the interaction of local anesthetics with Ca^{2+} showed that procaine partially mimics Ca^{2+} effect [2], [6], [14] since between these two agents there is a competition for the same sites in the membrane which may be occupied according to the ratio of their concentrations [4], [11], [14], [18], [20]. It was also shown that when external Ca^{2+} concentration is increasing a hyperpolarization of the membrane takes place [8], [9], [12] whereas decreasing its concentration results in a depolarization [5], [7], [13], [19].

In a previous paper the study of procaine effect on the membrane in free Ca^{2+} medium [14] showed that the anesthetic decreased the depolarization induced by the absence of the ion. In order to extend the observations regarding the dependence of procaine action on the external Ca^{2+} concentration, the phenomena in high Ca^{2+} medium have been investigated in the present work.

MATERIAL AND METHODS

The experiments were performed on frog (*Rana ridibunda* Pall.) sartorius fiber membrane using the glass intracellular microelectrode method. In each experiment five muscles were used at room temperature. Bicarbonate buffer solution at pH = 7.2 and phosphate buffer solution at pH = 6.0 have been used. The solutions with 5 mM Ca^{2+} were prepared by substitution of an equimolar quantity of NaCl for $CaCl_2$ and those with procaine (2.5 mM and 1 mM) by adding the anesthetic to Ringer solution. Statistical significance was calculated by Student's test.

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RESULTS

The normal resting potential had values between 93.64 mV and 94.30 mV (SE about 0.50 mV) (Figs. 1—4 : RP).

In control experiments the membrane potential was recorded in Ringer with 5 mM Ca^{2+} at pH = 7.2 and 6.0. The increment of external

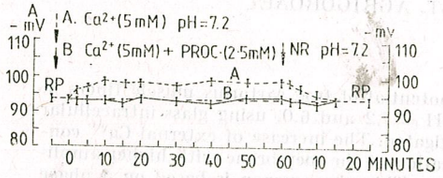

Fig. 1. — The effect of 5 mM Ca^{2+} and 2.5 mM procaine on the membrane potential, at pH = 7.2.
A : 5 mM Ca^{2+}-Ringer
B : 5 mM Ca^{2+} and 2.5 mM procaine—Ringer

Ca^{2+} concentration determined a hyperpolarization of the membrane, reversible in normal Ringer (NR), both in alkaline medium (Fig. 1 line A) and in acid medium (Fig. 3 A). At pH = 6.0 the average amplitude

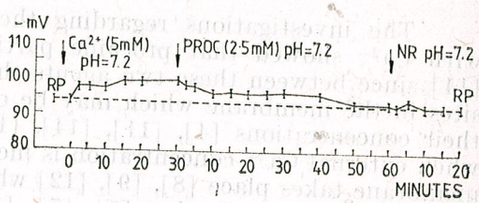

Fig. 2. — The effect of 2.5 mM procaine on the membrane potential after the action of 5 mM Ca^{2+}, at pH = 7.2.

of this phenomenon was higher than at pH = 7.2 (5.69 mV *versus* 4.41 mV), but its velocity was smaller.

The effect of procaine on membrane potential in high Ca^{2+} Ringer was followed at pH = 7.2 and 6.0. In a first series of experiments, in alkaline medium the presence of both 2.5 mM procaine and 5 mM Ca^{2+}

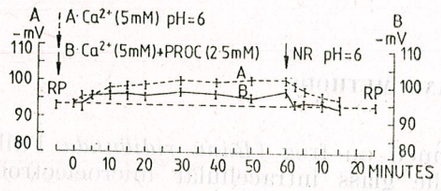

Fig. 3. — The effect of 5 mM Ca^{2+} and 2.5 mM procaine on the membrane potential, at pH = 6.0.
A : 5 mM Ca^{2+}-Ringer
B : 5 mM Ca^{2+} and 2.5 mM procaine—Ringer

(Fig. 1—B) did not modify the membrane resting potential ,whereas when added separately each one produced a hyperpolarization [2]. When 2.5mM procaine acted on a membrane previously hyperpolarized by 5 mM Ca^{2+} (Fig. 2), the hyperpolarization decreased very slowly to the level of resting potential value. At pH = 6.0 the presence of both 2.5 mM procaine and 5 mM Ca^{2+} (Fig. 3—B) in the medium brought about a reversible hyperpolarization of the membrane with a lower magnitude (3.33 mV)

than that produced by 5 mM Ca^{2+} alone (5.69 mV) at the same pH value. When a concentration of 1 mM procaine was used, which at this pH produces a slight depolarization of the membrane [2], and 5 mM Ca^{2+}, a membrane hyperpolarization (Fig. 4—B) with a smaller amplitude (2.46 mV) than in the previous case was recorded.

Therefore, a reduction of Ca^{2+} effect by procaine is observed, more pronounced in alkaline than in acid medium.

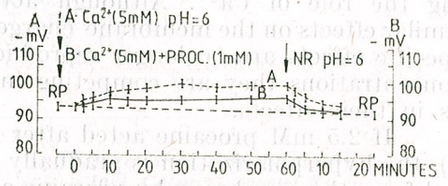

Fig. 4. — The effect of 5 mM Ca^{2+} and 1 mM procaine on the membrane potential, at pH = 6.0.
A : 5 mM Ca^{2+}-Ringer
B : 5 mM Ca^{2+} and 1 mM procaine—Ringer.

DISCUSSIONS AND CONCLUSIONS

In our previous experiments it was observed that by decreasing Ca^{2+} concentration in the external medium a depolarization of the membrane was recorded [14], with a slower onset, a lower amplitude and a faster recovery to the initial value in normal Ringer as compared to the depolarization determined by high external K^+ [15].

The change of $K^+ : Ca^{2+}$ ratio by increasing Ca^{2+} concentration brings about a hyperpolarization of the membrane (Fig. 1—A and Fig. 3—A) with rapid installation, low magnitude and slow recovery in normal Ringer.

The explanation of our results is based on "2—M.S.I." concept [1] on the membrane structure and properties, considering that there is an antagonism between K^+ and Ca^{2+} at the membrane phospholipidic structure level, point of view asserted by other authors too [10], [21]. Thus, the hyperpolarization by high external Ca^{2+} (as well as that produced by low external K^+) might be explained by a phase transition of globular phospholipidic micellae from the external layer of the membrane to laminar micellae, by replacing K^+ by Ca^{2+}, which is in excess. The characteristic aspects of these phenomena are a result of the fact that in different situations in phase transitions are involved phospholipidic micellae varying in number and sensitivity toward structural and destructural ions [14]. Our data bring new proofs in this respect. At an acid pH (Fig. 3—A) a higher membrane hyperpolarization was observed, but slower than at an alkaline pH (Fig. 1—A). This fact might be explained on the basis of a specific effect of the acid pH at the level of globular micellae from the external layer of the membrane [14], [15] on which the destructural ion (Ca^{2+}) is acting.

The investigations regarding the interaction of procaine with Ca^{2+} [4], [11], [18], [20] showed that the anesthetic is able to substitute Ca^{2+} from the membrane or is substituted by it, according to the ratio of their concentrations. In a free-calcium medium [14], [17], [19] pro-

caine diminished the depolarization induced by the absence of this ion, due to its ability to mimic the role of membrane Ca^{2+}, stronger at pH = = 6.0 [14].

In an alkaline medium containing 5 mM Ca^{2+} and 2.5 mM procaine, an antagonism is manifested between these two agents which results in a complete blocking of membrane hyperpolarization (Fig. 1—B). In a previous paper [2] we reported that 2.5 mM procaine in normal Ringer determined a slight hyperpolarization of the membrane partially imitating the role of Ca^{2+}. Although acting separately both agents have similar effects on the membrane charge, when acting simultaneously their specific effects are no longer reproduced. Probably at this ratio of their concentrations they are competing in binding to the membrane as well as in their effects.

If 2.5 mM procaine acted after the installation of Ca^{2+} effect (Fig. 2), the hyperpolarization is gradually diminished as a result of ion removal from the membrane by procaine and of a reduced ability of the anesthetic to mimic Ca^{2+} role in these conditions.

To appreciate procaine — Ca^{2+} interaction at acid pH one should consider that this pH causes certain specific aspects of the phenomena: an enhancement of hyperpolarization by high external Ca^{2+}, a preponderance of the cationic form of procaine [2], [3] and some modifications of globular phospholipidic micellae from the external layer of the membrane [2], [10], [15]. At pH = 6.0 a reduction of procaine efficiency in competing with Ca^{2+} is observed. Thus, the decrease of hyperpolarization is smaller than at an alkaline pH, at the same concentration of the anesthetic (2.5 mM) (Fig. 3—B). In the case of depolarizing concentration (1 mM) of procaine [2] the effect of simultaneous action of these two agents (Fig. 4) is no longer based on an antagonism, but rather due to a sum of their individual effects. The degree of reciprocal reduction of the two opposite effects is dependent upon the concentration ratio of the two agents.

It is evident that at an alkaline pH the procaine efficiency in antagonizing the hyperpolarizing effect of high Ca^{2+} is more marked than at acid pH.

Our data are in good agreement with the concept that the interaction between local anesthetics and Ca^{2+} occurs at the level of membrane phospholipidic structures [4], [18], [20]. Although there have been some other explanations [11], [16] we believe that the interaction between local anesthetics and calcium occurs at this level.

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BIOSYNTHESIS OF NICOTINAMIDE ADENINE DINUCLEOTIDE IN THE FROG LIVER. EFFECT OF NICOTINAMIDE AND NICOTINIC ACID ADMINISTRATION

BY

R. MEŞTER, D. SCRIPCARIU and A. FIXMAN

Single injection of nicotinamide (0.5, 1 and 2 mg per g body weight) has been shown to cause an increase in the NAD content of the frog liver. Increased synthesis of total NAD reached a maximum after 12 hours (500 % increment at 1 mg nicotinamide) and affected similarly both NAD^+ and NADH levels. Liver NAD concentration remained high for at least 24 h after a single injection (200 % at 0.5 mg, 300 % at 1 mg and 450 % at 2 mg nicotinamide), above the values in control animals. Nicotinic acid also increased total NAD content in the frog liver (3 fold at 12h after 0.5 mg per g body weight). It is concluded that both precursors participate similarly in the biosynthesis of NAD in the frog liver.

Nicotinamide and nicotinic acid are the main metabolic precursors used by the tissues in the biosynthesis of nicotinamide adenine dinucleotide (NAD). Biosynthesis of NAD from nicotinamide and nicotinic acid was elucidated by Preiss and Handler [13], Dietrich *et al.* [1], Ikeda *et al.* [5]. Nicotinamide stimulates the synthesis of NAD in some mammalian tissues : hepatic tissue, cardiac muscle, mammary glands and thyroid gland [2], [3], [4], [11]. Nicotinic acid has also proved to be a precursor of NAD in the hepatic tissue, the thyroid gland and erythrocytes [5], [9], [12]. The importance of each pathway is difficult to evaluate in biological systems. Although the effects of nicotinamide mononucleotide (NMN) on rats may be the main system in the liver, other studies have shown that the nicotinic acid also induces an increase in the concentration of NAD [6]. Injection of nicotinamide and nicotinic acid induces a transient increase in the level of NAD in the liver of mouse and rat [7], [8] and thyroid gland [11]. The pathways of NAD synthesis in lower vertebrates is not understood. A previous study in our laboratory has shown the presence of an active NMN adenylyltransferase in the oocytes and during the first stages of embryonic development of frog, suggesting the importance of nicotinamide in the biosynthesis of NAD [14].

The purpose of this paper is to present evidence that both nicotinamide and nicotinic acid, administered *in vivo*, are used as precursors of NAD synthesis in the frog liver.

MATERIAL AND METHODS

Rana ridibunda frogs from the lakes around Bucharest were used. Frogs weighing 100—150 g were injected with 1 ml of precursors, prepar-

ed in physiologic saline solution. Administration of nicotinamide and nicotinic acid was made by injection into the dorsal lymph sacs of animals. The following concentrations of nicotinamide were used : 0.5 mg, 1 mg and 2 mg per g body weight. Nicotinic acid was used only in a concentration of 0.5 mg per g body weight, higher concentrations having toxic effects upon the animals. The level of NAD was also determined on control animals.

Extraction of oxidized NAD. 1 g liver was homogenized with 4 ml trichloroacetic acid 2.5% at cold. The homogenate was centrifuged at 10.000 g for 10 min. The extracts were neutralized to a neutral pH (7.1—7.2) with 0.5 N Na_3PO_4 and used for experiment. The method was essentially the same as that earlier described [18].

Extraction of NADH. A procedure described by Telepneva and Meșter [18] was used for the extraction of reduced pyridine nucleotide. Briefly, 1 g hepatic tissue was homogenized in 5 ml 1 N KOH, prepared in ethanol 25%. The homogenate was boiled at 100°C for 2 min and then cooled on ice. After cooling down, the homogenate was neutralized with 1 M malic acid. The solution was clarified by centrifugation at 10.000 g for 10 min and used for experiments.

The method employed for the determination of NAD^+ and NADH was the same as that described by Slater *et al.* [16].

RESULTS AND DISCUSSION

NAD content in normal frog liver. The mean values of NAD^+ and NADH concentrations in the normal hepatic tissue of frog are summarized in Table 1. From the above data, it appeared that the pyridine nucleotide content of the frog liver is lower in comparison with that of mammals [6], [18]. The total NAD content is 43 μg/g of wet tissue, a value ten times smaller than that of the rat liver. This would be expected since the amphibian tissues generally have lower enzyme activities. Nevertheless, similar to the mammalian tissues, the level of oxidized NAD in frog liver is 4 times higher than the NADH content.

Table 1

Nicotinamide adenine dinucleotide concentration in frog liver (mean values of 10 experiments)

	No. of animals	NAD^+ μg/g tissue	NADH μg/g tissue	total NAD	NADH/NAD^+
Frog liver	10	32.6	9.3	42.9	0.28

Biosynthesis of NAD in vivo. In order to follow, *in vivo*, the synthesis of NAD, four lots of frogs were treated with different nicotinamide and nicotinic acid concentrations. After a single dose of precursor injection, the animals were sacrified at different time intervals (3, 6, 12 and 24 hours) and the concentrations of oxidized and reduced forms of pyridine nucleotide were determined. Each result represents the mean value of three determinations.

The nicotinamide injection in the tested concentrations significantly activates the NAD^+ and NADH levels of the hepatic tissue (Figs. 1 and 2). The highest concentrations of NAD^+ and NADH were established 12 hours after the nicotinamide injection. It is interesting to note that all the nicotinamide concentrations tested yield a highest activation of the pyridine nucleotide synthesis at the same time interval. In comparison with the control animals, 12 hours after the precursor administration, the NAD^+ level in the liver increased by 300% at 0.5 mg nicotinamide, by 530% at 1 mg nicotinamide and by 500% at 2 mg nicotinamide. It should be noted that the NADH content, 12 hours after nicotinamide injection, increased proportionally to those obtained for oxi-

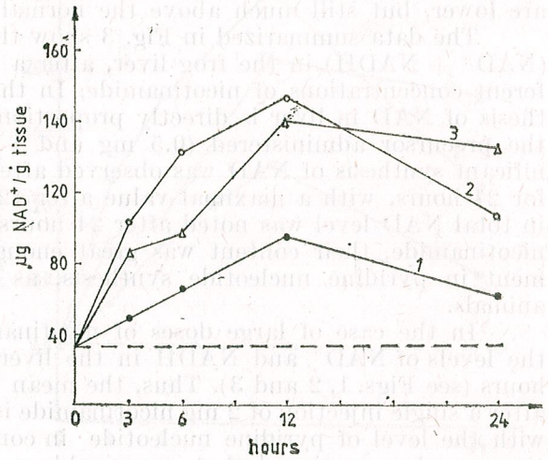

Fig. 1. — Effect of a single injection of nicotinamide in a concentration of 0.5 mg/g body weight (1), 1 mg/g body weight (2) and 2 mg/g body weight (3) on oxidized NAD concentration from the frog liver (mean values of three series of experiments). The dotted line indicates the level of NAD^+ in the liver of control frogs (mean values of 10 experiments).

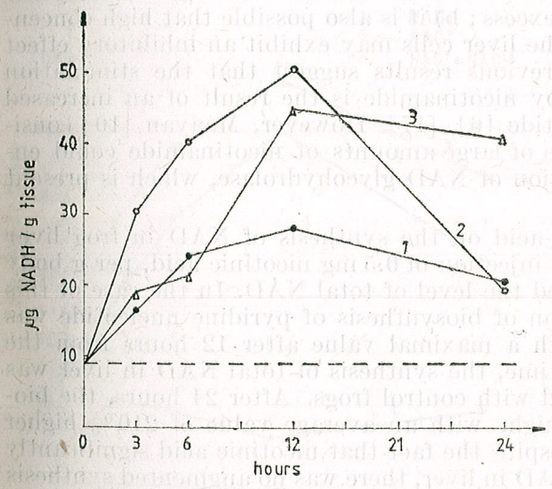

Fig. 2. — Effect of a single injection of nicotinamide in a concentration of 0.5 mg/g body weight (1), 1 mg/g body weight (2) and 2 mg/g body weight (3) on NADH concentration from the frog liver (mean values of three series of experiments). The dotted line indicates the content of NADH in the liver of control frogs (mean values of 10 experiments).

dized NAD. In other words, nicotinamide injection *in vivo* stimulated the synthesis of both oxidized and reduced forms of pyridine nucleotide (see Figs. 1 and 2).

The highest mean values of NAD^+ and NADH were obtained after administration of 1 mg nicotinamide. 24 hours after administration of 0.5 mg and 1mg nicotinamide, the concentrations of NAD^+ and NADH are lower, but still much above the normal limits.

The data summarized in Fig. 3 show the profile of total NAD level (NAD^+ + NADH) in the frog liver, after a single injection of three different concentrations of nicotinamide. In the first 12 hours, the biosynthesis of NAD in liver is directly proportional with the concentration of the precursor administered (0.5 mg and 1 mg nicotinamide). The significant synthesis of NAD was observed after 3 hours and was continued for 24 hours, with a maximal value after 12 hours. Although a decrease in total NAD level was noted after 24 hours for the 0.5 mg and 1 mg of nicotinamide, their content was great enough to account for the increment in pyridine nucleotide synthesis, as compared with the control animals.

In the case of large doses of nicotinamide (2 mg/g body weight), the levels of NAD^+ and NADH in the liver remain high, even after 24 hours (see Figs. 1, 2 and 3). Thus, the mean value of total NAD 24 hours after a single injection of 2 mg nicotinamide is 440% higher, in comparison with the level of pyridine nucleotide in control frogs. Several possibilities may be mentioned that conceivably could account for this enhancement : a) the biosynthesis of NAD is maintained at a high level by the presence of the precursor in excess ; b) it is also possible that high concentrations of nicotinamide in the liver cells may exhibit an inhibitory effect of NAD glycohydrolases. Previous results suggest that the stimulation of NAD synthesis induced by nicotinamide is the result of an increased synthesis of pyridine nucleotide [6], [17]. However, Maayan [10] considered that the administration of large amounts of nicotinamide could enhance NAD level, by inhibition of NAD glycohydrolase, which is present in the thyroid gland.

The effect of nicotinic acid on the synthesis of NAD in frog liver is presented in Fig. 4. Single injection of 0.5 mg nicotinic acid, per g body weight, significantly increased the level of total NAD. In the case of this precursor too, the stimulation of biosynthesis of pyridine nucleotide was observed after 3 hours, with a maximal value after 12 hours from the precursor injection. At this time, the synthesis of total NAD in liver was by 270% higher as compared with control frogs. After 24 hours, the biosynthesis of NAD remains high, with an average value of 210% higher than the control animals. Despite the fact that nicotinic acid significantly increased the level of total NAD in liver, there was no augmented synthesis of the reduced form of pyridine nucleotide. This aspect is difficult to appreciate, as experimental data are missing. It is possible that nicotinic acid may affect the reduction of NADH in liver cell (stimulation of NADH oxidation) or the activity of the enzymes which are NADH dependent.

Our experimental data are in agreement with those obtained on some mammalian tissues. High concentrations of nicotinamide have been shown to cause an increase of mouse liver NAD, the maximal content

being reached 8 hours after injection [7], [8]. Maayan and Rosenberg [11] reported that four hours after injection of nicotinamide, NAD content of rat thyroid was about 200% increased ; the level of NAD was

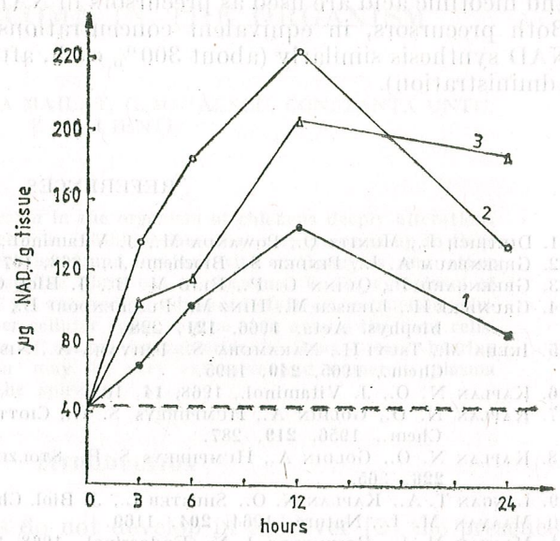

Fig. 3. — Effect of a single injection of nicotinamide in a concentration of 0.5 mg/g body weight (1), 1 mg/g body weight (2) and 2 mg/g body weight (3), on total NAD content from the frog liver. The dotted line indicates mean values of total NAD content in the liver of control animals (10 experiments).

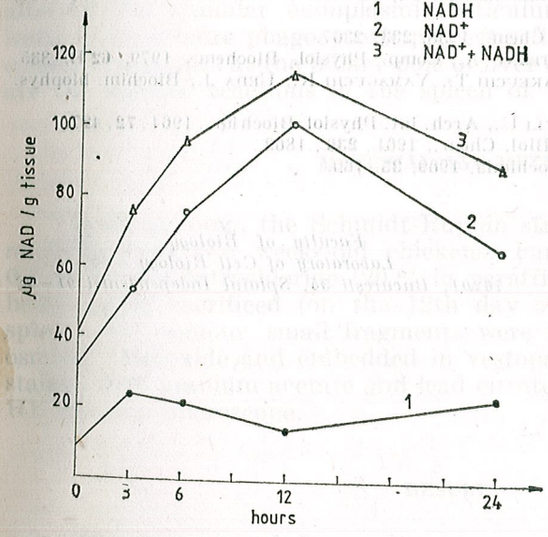

Fig. 4. — Biosynthesis of NAD in the frog liver after administration *in vivo* of nicotinic acid in a concentration of 0.5 mg/g body weight. Effect upon the level of NADH (1), NAD^+ (2) and total NAD concentration (3) in frog liver (mean values of three series of experiments).

little affected by nicotinic acid. However, Shimoyama *et al.* [15] demonstrated that both nicotinamide and nicotinic acid may act as precursors of NAD synthesis in the early embryonic stages, during chick embryonic development.

CONCLUSIONS

The results obtained by us presented evidence that nicotinamide and nicotinic acid are used as precursors in NAD biosynthesis by frog liver. Both precursors, in equivalent concentrations (0.5 mg), have increased NAD synthesis similarly (about 300% each, after 12 hours of a single dose administration).

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CHICKENS LIVER CELLS IN THE PRESENCE OF ROUS SARCOMA IN THE ORGANISM

BY

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In the presence of *Rous sarcoma* in the organism of chickens deeply alterations develop in the liver. The liver cells exhibit lesions that may progress up to their disaggregation. The cell debris are found together with erythrocytes and other elements of sinusoids. Collagen-like fibres can be discerned in the cytoplasm of the liver cells that have not yet reached an advanced state of destruction. Numerous plasma cells and other cellular types can be seen among the liver cells. Following the administration of carbon tetrachloride to Rous tumour-bearing chickens, hepatic destruction may be very extensive and numerous plasma cells are differentiated in the spleen.

INTRODUCTION

Even when metastases do not develop in the liver [2] the presence of tumours in the organism has a marked influence upon the liver cells, altering the granular endoplasmic reticulum and mitochondria which leads to an intense phagocytosis process reflected by the presence of secondary lysosomes at the biliary pole of liver cells ([4], [8]). More frequent are the marked reactions in the spleen of tumour-bearing animals [7].

MATERIAL AND METHOD

Rous sarcoma, the Schmidt-Ruppin strain, was transplanted intramuscularly to two weeks-old chickens. Part of the chickens received 0.5 ml carbon tetrachloride (20 % in paraffin oil), "per os", three days before being sacrificed (on the 12th day after transplantation). Liver, spleen and tumour small fragments were fixed in glutaraldehyde and osmium tetraoxide and embedded in vestopal. The sections were double stained with uranium acetate and lead citrate and examined in a Hitachi-HU electron microscope.

RESULTS

The presence of Rous sarcoma tumour (VSR, Schmidt-Ruppin strain) profoundly disturbs the life of liver cells, and hence, causes striking ultrastructural alterations up to the destruction of cells.

In most of the cells that maintain their integrity (even if only apparently) the nuclei are considerably diminished in size, sometimes deeply notched, with an altered content and little compact chromatin disseminated peripherally (Fig. 1). In the incipient phases of hepatocytes transformation, collagen-like fibres may be observed in their cytoplasm.

While the cells are in the course of disruption their nuclei still maintain an almost normal shape, structure and size. Particularly worthy of note is the behaviour of glycogen which is not eliminated as happens in most instances when the organism is stressed by different chemical or physical factors or diseases. The glycogen granules of normal aspect are distributed especially in the cytoplasmic areas free of mitochondria, the granules being sometimes slightly agglutinated.

In some parts of the cytoplasm of the cells that have reached an advanced degree of alteration a partial lysis of glycogen granules may nevertheless be seen. These zones become clear since the fine cytoplasm granulations also disappear and the glycogen granules, marked by lysis, are randomly distributed throughout these spaces in the cytoplasm (Fig. 1). These zones are the site at which the liver cells breakdown in the more advanced stages of destruction.

The mitochondria, so numerous in hepatocytes, exhibit a wide range of alterations. Most of them are transformed into spherules full of granulations that render them more or less electron-dense. Many of them are surrounded by cisternae of the granular endoplasmic reticulum, which are disrupted to various extents but always equal to the degree of disaggregation of the mitochondria it surrounds (Fig. 1).

In some cases, even when disruption is very advanced, the mitochondria may maintain the traces of cristae, marked by electron-clear linear spaces.

When their rim can no longer be discerned, the mitochondria lose their identity and merge with the cytoplasm, with its glycogen and ribosomal granules of the granular endoplasmic reticulum that surrounded the mitochondria. A kind of vacuolization marked by electron-clear spaces can be discerned in the mitochondria in very few cases. In the completely destroyed cells or in the lumen of the sinusoid capillaries with altered walls, the mitochondria appear to maintain their external membrane and to be adrift (Fig. 2).

Disruption of sinusoid capillary walls is accentuated (Fig. 2). Through the gaps in the endothelial cells, the cytoplasm of the brokendown hepatocytes penetrates into the lumen of capillaries and its glycogen granules, mitochondria, etc. can be seen in the neighbourhood to the red blood cells in the capillaries (Fig. 2).

The presence of plasma cells among hepatocytes offers an additional argument demonstrating the strong reaction of the organism and of the liver to the presence of Rous sarcoma. The liver cells are no longer rich in endoplasmic reticulum cisternae, that is a particularity of these cells; the cisternae are characteristically dilated and come in direct contact with the clear space of the nuclear membrane (Fig. 3). No plasma-

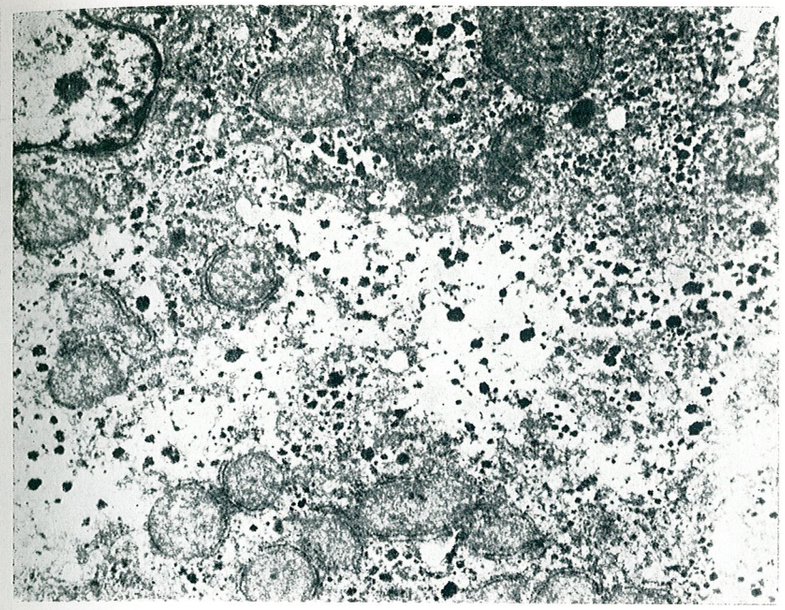

Fig. 1. — Cytoplasmic areas in which partial glycogen lysis has occurred. The mitochondria still maintain their contour. × 18.500

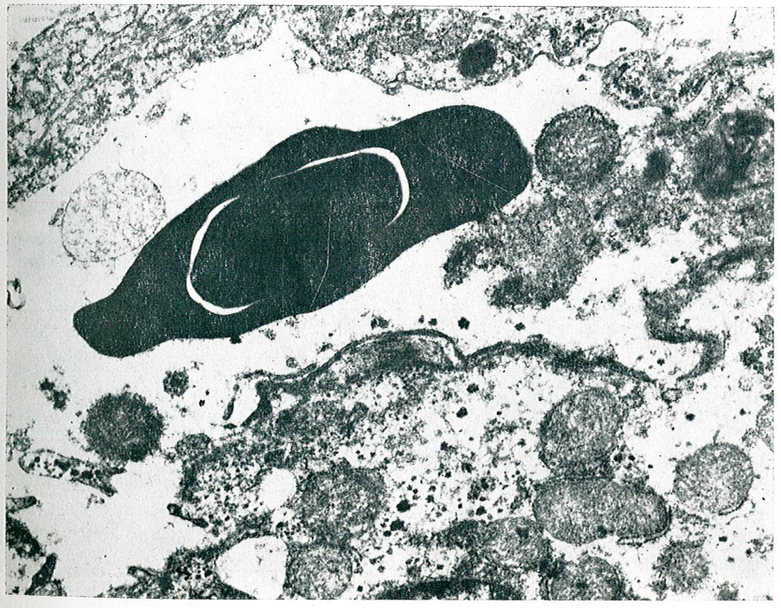

Fig. 2. — Disrupted hepatocytes and sinusoid capillary cells. × 11.900

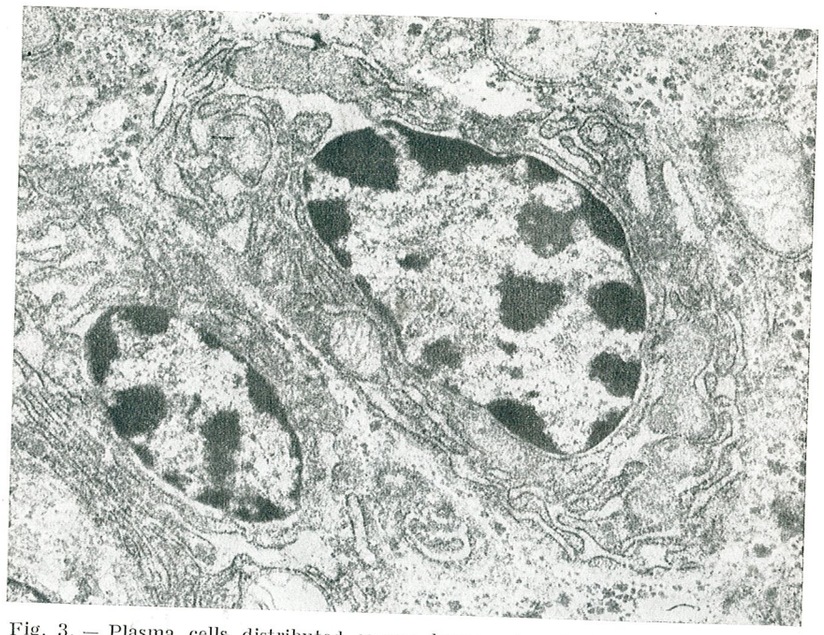

Fig. 3. — Plasma cells distributed among hepatocytes in Rous sarcoma-bearing chickens. × 8.000

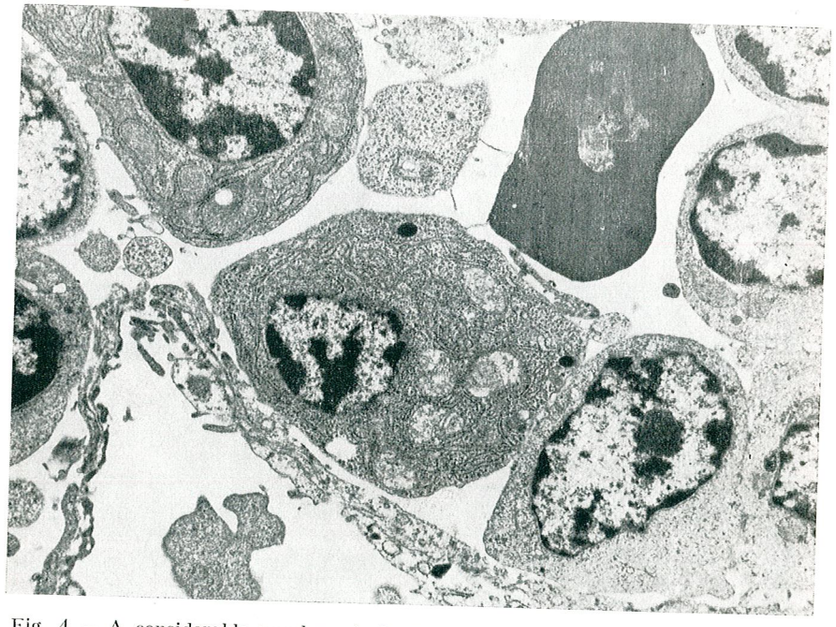

Fig. 4. — A considerable number of plasma cells in the spleen of Rous sarcoma-bearing chickens treated with carbon tetrachloride. × 6.200.

lemma belonging either to plasma or to liver cells can be discerned at the site of contact between these two types of cells.

Following the administration of carbon tetrachloride to Rous sarcoma-bearing chickens, hepatic destruction is more marked, with the presence of erythrocytes in the liver cells, and in the spleen the differentiation of very numerous plasma cells can be seen (Fig. 4).

DISCUSSION AND CONCLUSIONS

Marked alterations are produced in certain areas of the liver in the presence of Rous sarcoma in the organism.

We believe that the collagen-like fibres that appear in the less affected hepatocytes cannot be considered as fibres associated with desmosomes as assumed in certain cases of deterioration of liver cells [12].

Growth of Rous sarcoma in the organism of chickens causes a greater deterioration of the liver cells than that produced by ascites hR-18 in the white rats [8] or by melanoma H-16 in the hamster [4]. The destruction may lead to disaggregation as in most cases of administration of some toxical substances [5], [6], [9], [10], [12].

Destruction of the hepatic parenchyma does not resemble that caused by metastases [2].

The presence of plasma cells and other cellular elements has often been noted [1]. Under our experimental conditions plasma cells were to be found among the hepatocytes in the vicinity of sinusoids.

Following the administration of carbon tetrachloride, disruption of the liver parenchyma is more accentuated [5], [10], [12] and the spleen exhibits a considerable number of plasma cells, the reaction being more marked than in the Rous sarcoma chickens not treated with carbon tetrachloride. The presence of the tumour induces the occurrence of plasma cells in the spleen [7], but the reaction is less important than that of plasmacytes of Rous sarcoma-bearing animals treated with carbon tetrachloride [3], [11].

The marked disruption of chicken hepatocytes may also be attributed to Rous sarcoma virus that accentuates the perturbed physiology of the tumour-bearing animal.

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THYMIC REACTIONS IN THE TUMOURS BEARING RATS

BY

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The experiments performed on male Wistar rats have shown an involution of thymus proved also histologically by a decreased weight, concomitantly with the increase of Walker 256 tumoral transplant. The thymus involution was delayed by the subsequent adrenalectomy of these rats.
In parabiotic rat-pairs in which one partner was inoculated with ascite OYA, the phenomenon of thymic involution was identified in both animals, probably because of tumour toxohormone migration.

Earlier papers published by one of us [5], [7] showed that Wistar rats inoculated with Walker 256 carcinosarcoma produced a constant progressive and irreversible thymic involution.

The phenomenon can be considered as a symptom of breakdown of antitumoral organism reactivity as well as a stress reaction, the gland involution being diminished by an adrenalectomy of tumour bearing animals.

This paper presents some results of our investigations on the effects of some cancer inducing transplant variants on the thymus function in the cellular immunity reactions as well as in antitumoral organism defense [6].

MATERIALS AND METHODS

1) Young one day old rats were subcutaneously inoculated in the interscapulary region with Walker 256 carcinosarcoma. After tumour transplantation, the animals were left with their mothers and sacrificed weekly, during a month, the thymus being weighted at a torsion balance. The control groups undergoing a false operation were kept in the same conditions.

2) Male rats weighing 100 $\pm$ 10 g were transplanted with Walker 256 tumour. The animals were bilaterally adrenalectomized 2 or 4 weeks after grafting. Sacrification, prelevation and weighing of thymus were made 7 days after operation. All animals were kept under salts diet.

3) Pairs of 100 g male rats were united by musculo-cutaneous sutures according to Bunster-Meyer's parabiotic technique quoted by Dorfman [2]. It is of importance to have the two members of a pair closely matched in weight. Three days after the surgical intervention, one of the partners was inoculated with 1 ml ascite OYA [4], containing 32,000

cells/ml. The control parabiotic pairs were left uninoculated. Two weeks after transplantation, the animals were sacrificed and the thymus weighted in both animals.

In some cases histological thymus investigations by hematoxylin-eosin staining were kindly made by Dr. G. Simu.

RESULTS AND DISCUSSION

First week following inoculation tumorogenesis increasing was histologically observed as a reticuloepithelial hyperplasia of thymus. The thymic involution was accentuated and after one month it appeared as a thymic dropping. Hyperplasia and hypertrophia of the reticuloepithelial cells is completed by relatively numerous syncitial cells, the differentiation of zones and appearance of some villosities with PAS-positive materials. At the same time, the mortality of animals increased.

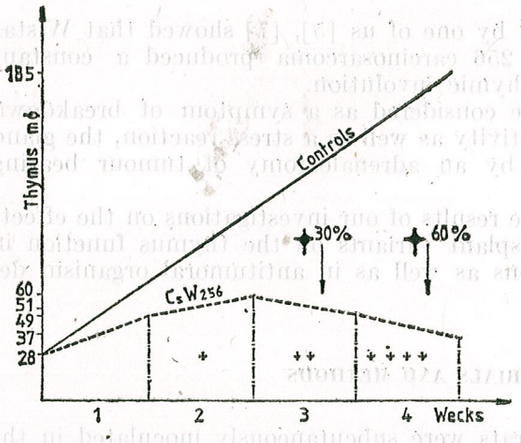

Fig. 1. — Dynamics of the thymus weight in Walker 256 carcinosarcoma bearing rats.

2) The adrenal eradication of rats, 2 weeks after Walker 256 carcinosarcoma grafting, when solid tumours appeared, produced a delay in thymic weight involution, compared with the non-adrenalectomized tumour bearing animals. Four weeks after grafting when the animals preented developed tumours, the thymus size was correspondingly decreased, with clear reticuloepithelial hyperplasia and increased Hassal corpuscles in the medular zone. At this time the thymic decrease could be diminished by adrenalectomy.

According to H. Selye's theory [8], thymus involution in the mentioned situations can be considered as a tumoral stress. The adrenal excision, i.e. of glucocorticosteroid thymolytic hormones, reduces, to some extent, thymic involution. In concordance with Blomberg [1], thymic involution can be interpreted as a removal of inert immunocompetent cells as well as an intensification of the T active lymphocytes with antitumoral role.

3) In the 16 parabiotic rat-pairs in which one partner was inoculated with 1 ml ascite OYA, it was observed that they generally can survive 2 weeks without presenting rejection phenomena. At the final stage of tumorogenesis, thymus weight of the inoculated animal diminished with 59.80 % compared with the values found at the uninoculated partner. The cortical zone appeared clear, the gland being formed of a mass of reticuloepithelial cells with numerous cystic PAS-positive structures. In the uninoculated partner of parabiotic rats, the thymus also decreased; the development of the structural zone details was inhibited, Hassal corpuscles were numerous and the fatty content was high. Henceforth, in both cases, the thymic involution was present, being more pregnant in the inoculated animals. Their parabiotic organism had a much more involuted thymus than the tumour-free pairs.

In this case we suppose that in the parabiotic partner the involution is provoked by the diffusion of a tumoral toxohormone through intersti-

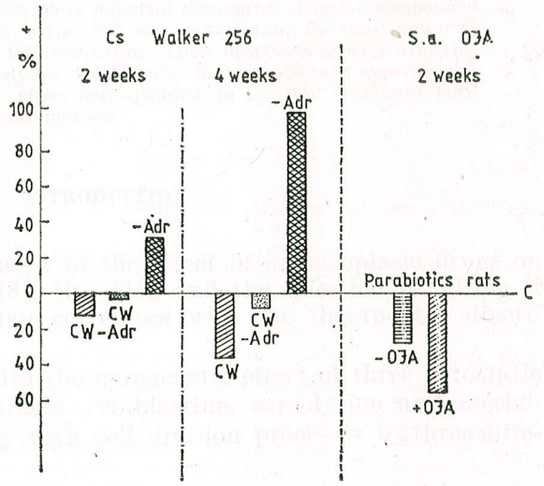

Fig. 2. — The thymus weight in rats bearing Walker 256 carcinosarcoma (CW); adrenalectomized (— Adr); adrenalectomized and tumor inoculated (—Adr+CW); and in the parabiotic rats bearing OYA sarcoma.

tial liquids as mentioned by Fukuoka and Nakahara [3]. This hypothesis is also supported by the fact that both animals died at the same time, showing general intoxication symptoms.

CONCLUSIONS

Malignant experimental tumours act as a stress factor which produces an irreversible involution of the thymus.

The phenomenon is generally dependent on the secretion of glucocorticosteroid hormone. This fact supports the idea that the thymus constitutes the link between the formation of specific immunological defense reactions and the nonspecific reactions of the organism.

Finally, the existence of a tumoral toxohormone with thymic involution action appears plausible.

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THE *IN VIVO* TIME-EFFECT RELATIONSHIP INDUCING CHROMOSOME ABERRATIONS BY THREE CYTOSTATIC DRUGS

BY

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The *in vivo* time-effect relationship in the yield of chromosome aberrations induced by vinblastine, sarcolysine and methotrexate on Guérin T_8 ascite cells was determined by comparing cytogenetic lesions consecutive to unique treatments applied 2, 4 and 6 days after ascite cells grafting. Chromosome observations were performed in the 8th and respectively the 30th day from grafting.
In the 8th day examination, the most powerful clastogenic effect corresponded to the last treatment time, and in the 30th day examination the most powerful effect corresponded to the first treatment time. These observations were available for both vinblastine and sarcolysine treatments. In methotrexate experiments, the most powerful clastogenic effect corresponded to the first treatment both in the 8th and the 30th day examinations.

INTRODUCTION

Many papers are dedicated to the effect of antineoplasic drugs on chromosomes [4], [5], [7], [8], [9], [10] and the question is raising if the clastogenic effect is or not correlated with the therapeutic effectiveness.

This paper is dealing with the cytogenetic effect of three cytostatic drugs of large clinical usefulness: vinblastine, sarcolysine and methotrexate, which are interfering with cell division processes by three different mechanisms.

MATERIAL AND METHOD

Tested drugs, vinblastine (0.3 mg/kg b.w.), sarcolysine (3 mg/kg b.w.) and methotrexate (5 mg/kg b.w.) were intravenously administered into Wistar rats, bearing Guérin T_8 ascite tumors. The treatments were performed in the 2nd, 4th and 6th day after intraperitoneally inoculation of 1×10^6 tumor cells/animal, and tumor cells fixation for cytogenetic examination was performed in the 8th and respectively the 30th day after ascite cells grafting. Light microscopy observations concerned the number and type of chromosome aberrations corresponding to each treatment and examination time. Also, for all tested drugs, a comparison between chro-

Table 1

Chromosomal aberrations induced by vinblastine, sarcolysine and methotrexate in Guérin T_8 ascite tumor cells of Wistar rats

Tested drugs	Examined (days from ascite grafting)	Treated (days from ascite grafting)	Total number of examined cells	Abnormal metaphases		"t" values (chromatid against chromosome types of aberrations)	Average survival time in days
				Observed	Expected		
Vinblastine (0.3 mg/kg b.w.)	in the 8th day	control	100	3	7.8	1.76	21.8
		2nd day	100	5	7.8	3.82 $P = 0.05$	27.1
		4th day	100	5	7.8	0	32.0
		6th day	100	18	7.8	1.02	21.8
	in the 30th day	control	50	2	5	0.144	21.8
		2nd day	50	7	5	2 $P = 0.05$	27.1
		4th day	50	9	5	3.137 $P = 0.05$	22.0
		6th day	50	2	5	2.13 $P = 0.05$	21.8
				$X^2 = 12.0$			
				$P \ll 0.01$			
Sarcolysine (3 mg/kg b.w.)	in the 8th day	control	100	4	24.5	1.005	22.7
		2nd day	100	15	24.5	1.69	24.7
		4th day	100	21	24.5	2.39 $P = 0.05$	31.6
		6th day	100	58	24.5	7.56 $P = 0.05$	30.1
				$X^2 = 67.142$			
				$P \ll 0.01$			
	in the 30th day	control	all animals died				22.7
		2nd day	50	10	9.7	1.15	24.7
		4th day	50	11	8.7	1.20	31.6
		6th day	50	8	9.7	2.13 $P = 0.05$	30.1
				$X^2 = 0.84$			
Methotrexate (5 mg/kg b.w.)	in the 8th day	control	100	2	16.7	—	18.5
		2nd day	11	9	1.8	—	28.0
		4th day	100	19	16.7	1.02	28.5
		6th day	100	22	16.7	3.05 $P = 0.05$	22.3
				$X^2 = 13.737$			
				$P \ll 0.01$			
	in the 30th day	control	all animals died				18.5
		2nd day	50	15	10.3	2.76 $P = 0.05$	28.6
		4th day	50	10	10.3	1.49	28.6
		6th day	50	6	10.3	0.46	22.3
				$X^2 = 3.947$			

mosome and chromatid types of aberrations has been made. The observed abnormalities were submitted to X^2 test, and also the "t" test was employed for comparing chromosome against chromatid types of aberrations.

RESULTS

From experimental data, summarized in Table 1, it could be seen that chromosomal aberrations induced by vinblastine, sarcolysine and methotrexate in Guérin T_8 ascite cells are of different incidence and type. The *in vivo* clastogenic effect of sarcolysine is greatly exceeding those of the other two tested drugs; methotrexate has induced an observable but moderate chromosome damage, followed by vinblastine whose cytogenetic action is weak. This aspect is evident considering the frequencies of abnormal metaphases, severity of induced lesions and the presence of multiple aberration cells.

The lapse of time between the ascite grafting and drug administration influences the clastogenic effectiveness of cytostatics tested and also, the examination time is very important for revealing the time-effect relationship (see Table 1). In the 8th day examination, the most powerful clastogenic effect corresponded to the 6th day treatment, and in the 30 th day examination, the most powerful effect corresponded to the 2nd day treatment. These observations were available for both vinblastine and sarcolysine treatments. In methotrexate experiments, the most powerful clastogenic effect corresponded to the first treatments either in the 8th or in the 30th day examinations.

DISCUSSIONS

It is interesting to observe that in the vinblastine and methotrexate treatments, the average survival time corresponding to the two earlier treatments (performed in the 2nd and respectively 4th day) is longer; on the contrary, in sarcolysine treatments, the two later administrations (performed in the 4th and in the 6th day after ascite grafting) were accompanied by a longer average survival time. Then, the aberrant cell ratios in tumor populations are not correlated with the average survival time, i.e. with the therapeutical effectiveness. The high frequencies of chromosome aberrations observed in the 8th day, corresponding to the vinblastine and sarcolysine treatments performed in the 6th day and not in those performed in the 2nd and the 4th day, could be due to some repair processes or to elimination by cell division of abnormal cells [1].

The three cytostatic drugs studied by us are interfering with cell division processes by three different mechanisms : blocking the cell spindle fibers synthesis [6], alkylation of DNA molecules, and, respectively, inhibition of some enzymatic pathways in the biosynthesis of DNA precursors [3]. Perhaps their different effect on chromosomes is due to the

action of these different and "possible independent inhibitory mechanisms" [2].

Then, chromosome aberrations could represent a "companion" of the cytostatic effect, but not a very important one.

Acknowledgement. We thank Grapina Vrabie, Mariana Papuc and Zinca Albu for their valuable technical assistance.

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EFFECT OF CHEMICALLY MODIFIED NYSTATINE IN ASSOCIATION WITH ACTINOMYCIN D AND PUROMYCIN ON HeLa CELL POLYSOMAL PROFILES

BY

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The effect of NsMC, a chemically modified nystatine preparation, on polysome structures in HeLa cells, administered either alone or in combination with actinomycin D and puromycin, was investigated. NsMC induces a reduction in polysomal material as compared to control polysome profiles. In combination with actinomycin D it enhances the activity of the latter due, probably, to an increase in cell permeability to this drug. In combination with puromycin it leads to a stabilization of polysome structures.

Polyene antibiotics are cytotoxic to sensitive cells due to their interaction with membrane sterols [3]. This leads to a permeabilization for intracellular compounds and eventually to cell death [4]. In a previous paper we showed that the same holds true for a chemically modified nystatine (NsMC) [1] with antitumor activity [6] at high concentrations (10 mg/ml) in the case of Guérin T-8 ascite cells. Much fewer data are available regarding possible intracellular effects on mammalian cells at lower concentrations. Changes in HeLa cell polysomal profiles, induced either by NsMC alone or in association with each actinomycin D and puromycin, are shown in the present paper.

MATERIALS AND METHODS

HeLa cells were grown in glass flasks until they formed a monolayer. Then fresh growth medium was added and after 24 hours the medium was changed for a medium containing either 2 mg/ml NsMC, 3 μg/ml actinomycin D, 3×10^{-4} M puromycin, or NsMC in combination with actinomycin D or puromycin, at the same concentrations. After incubating for 25 minutes, cleared cell lysates were prepared according to [10]. 0.5 ml lysate were layered on top of 10–50% sucrose gradients and spun at 36,000 rpm, at 2°C for 120 minutes in a Janetzki Vac-601 ultracentrifuge. The gradients were fractionated and read at 260 nm with the aid of a spectrophotometer. For each lysate three sucrose gradients were run.

RESULTS AND DISCUSSION

The NsMC concentration was chosen to be below its cytotoxic level in order to be able to follow up more subtle changes induced by the drug within the cell. At the concentration of 2 mg/ml culture media, NsMC does not exhibit an immediate cytotoxic action; nevertheless, it shows a pro-

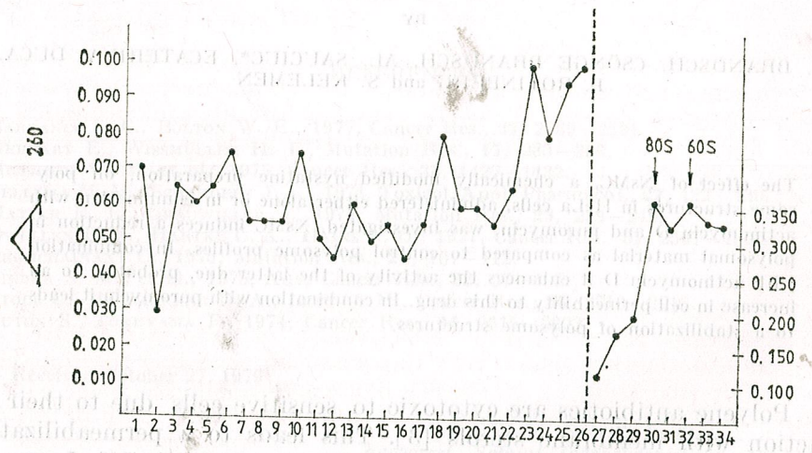

Fig. 1. — Polysome profile of control HeLa cells analysed on 10—50 % sucrose gradients, spun at 36,000 rpm at 2°C for 120 minutes.

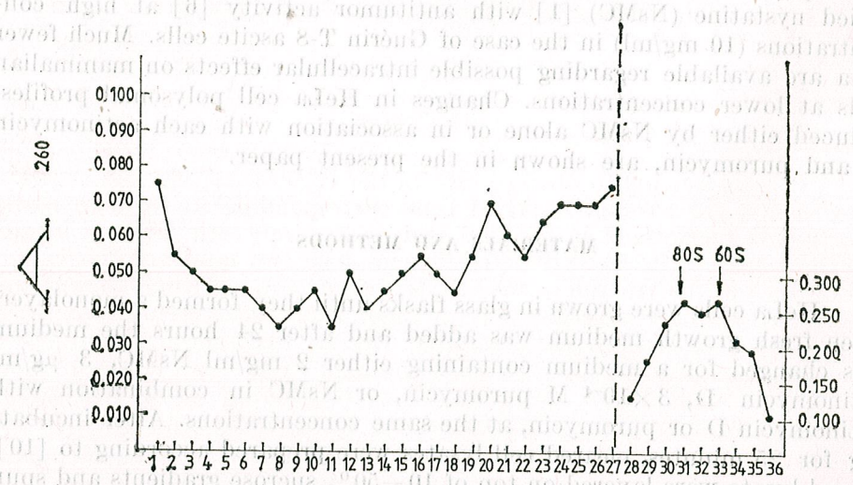

Fig. 2. — Polysome profile of NsMC treated HeLa cells. Same conditions as in Fig. 1.

nounced cytostatic effect within a 3 days period. The concentrations of actinomycin D and puromycin were chosen close to their activity limit on polysome structures [7], thus allowing the effect of associated treatment with NsMC to be observed.

Polysomal profiles, derived from NsMC treated HeLa cells, show a certain decrease in polysomal material and a shift of the polysomal peaks to the right, into the region of lighter polysomers (Fig. 2) as compared to control profiles (Fig. 1).

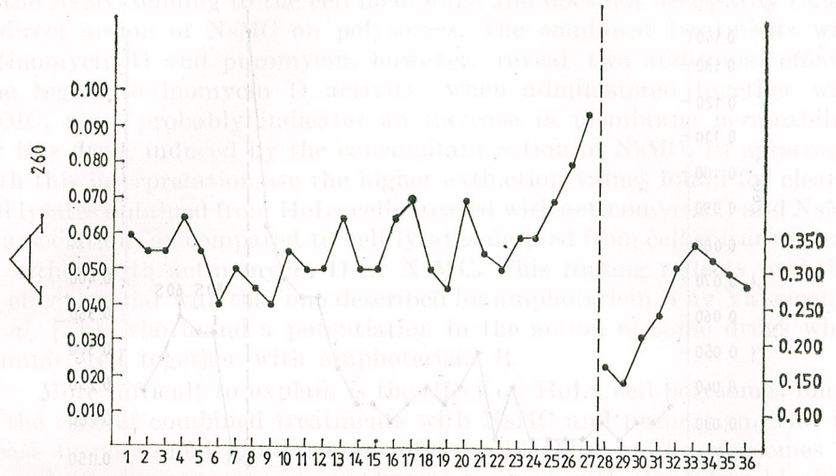

Fig. 3. — Polysome profile of actinomycin D treated HeLa cells, analysed on 10—50 % sucrose gradients. Same conditions as in Fig. 1.

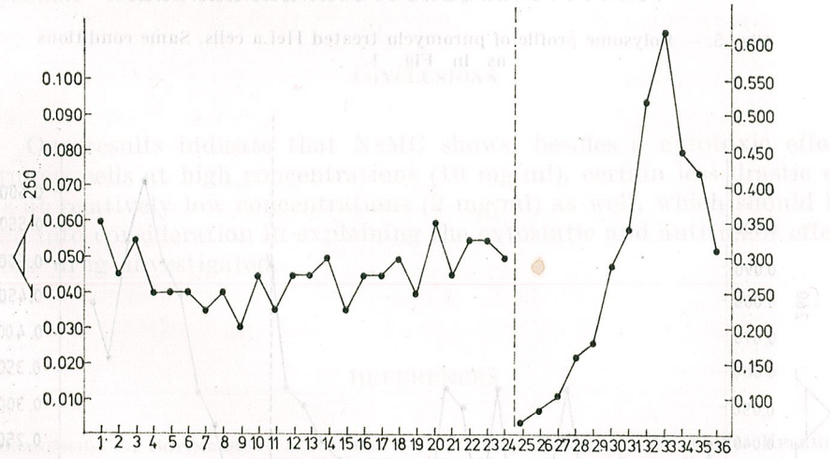

Fig. 4. — Polysome profile of HeLa cells treated with actinomycin D and NsMC. Same conditions as in Fig.1.

The polysomal profiles derived from cells treated with actinomycin D at a concentration of 3 μg/ml are very similar to those of control cells (Fig. 3). However, the combined treatment with actinomycin D and NsMC leads to an important reduction in polysomal material and a simultaneous increase of the peak corresponding to ribosomal subunits (Fig. 4).

Puromycin, at a concentration of 5×10^{-4}, leads within 25 minutes to the disorganization of the majority of polysomes (Fig. 5), but combined with NsMC a richer polysomal profile than in the case of the separate action of each drug is found (Fig. 6).

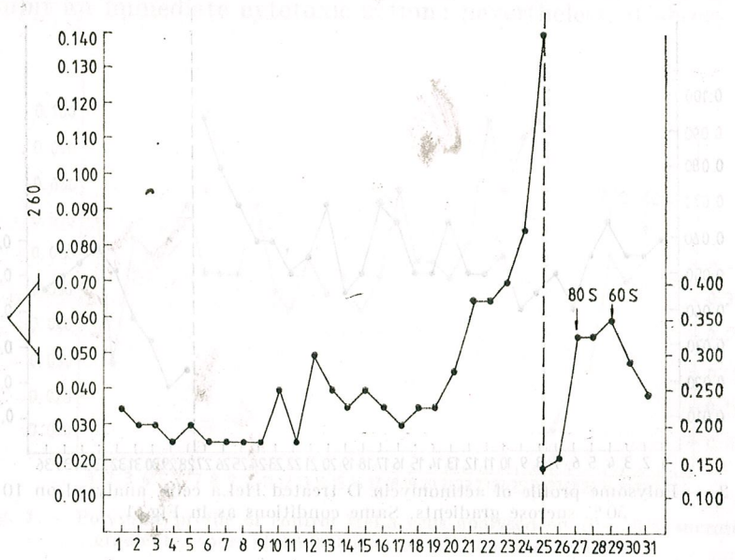

Fig. 5. — Polysome profile of puromycin treated HeLa cells. Same conditions as in Fig. 1.

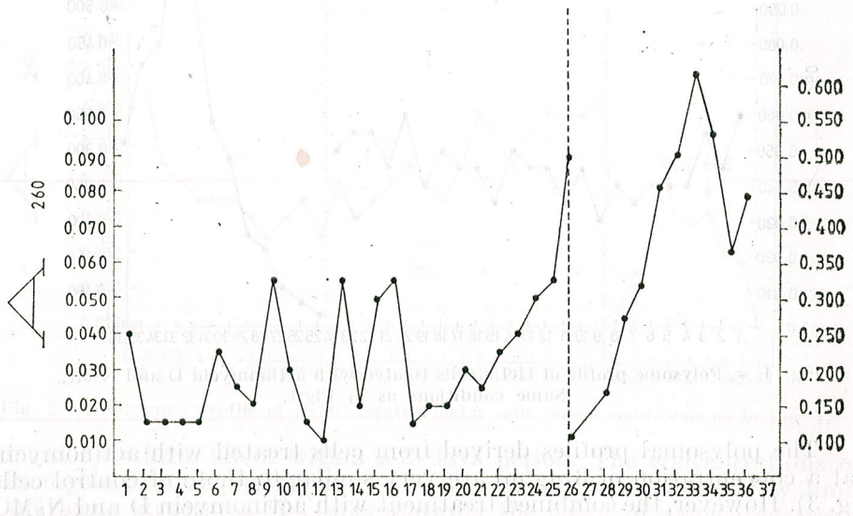

Fig. 6. — Polysome profile of HeLa cells treated with NsMC and puromycin. Same conditions as in Fig. 1.

The reduction in the amount of polysomal material and its shift to lower densities, that is to polysomes characterized by a smaller number of ribosomes attached to messenger RNA, following incubation of HeLa cells with NsMC, might be due to a secondary effect, occurring as a result of the NsMC binding to the cell membrane and does not necessarily reflect a direct action of NsMC on polysomes. The combined treatments with actinomycin D and puromycin, however, reveal two additional effects. The higher actinomycin D activity, when administered together with NsMC, most probably indicates an increase in membrane permeability for this drug, induced by the concomitant action of NsMC. In agreement with this interpretation are the higher extinction values found for cleared cell lysates obtained from HeLa cells treated with actinomycin D and NsMC in association, as compared to cell lysates derived from cell cultures treated either with actinomycin D or NsMC. This finding reflects probably an effect similar with that one described for amphotericin B by Yavemenko *et. al.* [11], who found a potentiation in the action of some drugs when administered together with amphotericin B.

More difficult to explain is the effect on HeLa cell polysomes found in the case of combined treatments with NsMC and puromycin. The increase in polysomal profiles may reflect a blocking of the ribosomes on the polyribosome structures as is known to occur with elongation blocking agents [2]. This would suppose an interaction of the two drugs at the polysomal level.

CONCLUSIONS

Our results indicate that NsMC shows, besides a cytotoxic effect on tumor cells at high concentrations (10 mg/ml), certain less drastic effects at relatively low concentrations (2 mg/ml) as well, which should be taken into consideration in explaining the cytostatic and antitumor effect of the drug investigated.

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ULTRASTRUCTURAL ASPECTS OF VACCINIA VIRUS MORPHOGENESIS IN THE L-23 LINE CELLS

BY

GR. MIHĂESCU, D. MIȘCALENCU, M. D. IONESCU

The ultrastructural study of vaccinia virus morphogenesis indicates two distinct ways in viral envelope construction. In the individual virion morphogenesis it is the internal envelope which is formed at the beginning and this closes gradually the viral components that will form the core. After that, the core is condensed. In the external envelope constitution the golgian cisternae are implicated. When the virions are assembled in the viral inclusion mass, all the viral envelopes are differentiated from inclusion materials, without golgian cisternae participation.

Vaccinia virion contains DNA-dependent RNA-polymerase that makes possible the DNA genome copying in viral mRNA still from viral core step existence, as the first phase of virion uncovering by hydrolytic lysosomal enzymes [9]. Viral mRNA codifies the synthesis of an early protein which determines DNA release from the viral core [1].

As a result, DNA and protein synthesis inhibition due to virion endonuclease takes place in the host cell. At the same time, cellular mRNA transfer to the cytoplasm is stopped, and along 2—3 hours from the moment of infection, only viral mRNA is synthesized.

The polysomes of the cell are subjected to breakdown and their materials are recycled for polysomal assembly by the viral mRNA [3] and a high intensification of viral DNA synthesis in the cytoplasm of the host cell takes place [5].

Electron microscopic studies regarding vaccinia virus morphogenesis have brought new information concerning the origin of viral envelope membranes.

The intention of the present paper is to underline some peculiar aspects of vaccinia virus morphogenesis, particularly regarding external viral envelope morphogenesis.

MATERIAL AND METHODS

L_{23}line cells from cell cultures laboratory of the Cantacuzino Institute have been growing on the IC 65 nutritive medium, supplemented with 2% calf serum. When the cellular monolayer was almost completely grown, we infected it with vaccinia virus. A double fixation with 2% glutaraldehyde and 1% osmium tetraoxide at 24 hours after infection has been performed.

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RESULTS

All the viral morphogenesis steps can be seen in the cell culture at 24 hours after infection. The rearranged structural areas called "viral factories" or viroplasma (Fig. 1) appear as the first morphological modification. Here the viral components are synthesized and the new constituted membranes will form the internal viral envelope.

Before the formation of mature viral particles, the vaccinia virus forms developmental bodies. They appear as isolated entities in the viral matrix, or are embedded in an electrondense mass which constitutes an inclusion.

Depending on their disposition (isolated or aggregated) we can distinguish two different ways of viral morphogenesis. Morphogenesis of isolated virions takes place in several phases :

1. the appearance of membranous profiles, *de novo* formed like calottes and hemispheres, that gradually close the viroplasmic material (Fig. 2) and will constitute the internal viral envelope. The Golgi apparatus is hypertrophied, but its role in this phase is unknown ;

2. the final phase of internal envelope morphogenesis and the beginning of viral core condensation (Fig. 3) ;

3. the viral core condensation and the appearance of an additional viral external envelope of Golgi cisternae origin, when the virions migrate to the centrosphere area of the cell (Fig. 4 a). The Golgi apparatus is hypertrophied (Fig 4 b). The similarity between Golgi membrane structure and membranous profile that covers virion is obvious (Fig. 5), without being detectable a direct cotinuity between them. The Golgi membrane gradually condensates on the virion surface and a mature virion with double envelope appears (Fig. 6). The second way of virion maturation is less common and corresponds to the formation of virions clustered in an electrondense material that constitutes an inclusion. It is characteristic that the genome is the first constituted structure, around which the remained viral components will be assembled. Morphogenesis is performed in the following steps :

1. the uncondensed genome composed of numerous very fine parallel fibrils surrounded by a fibrogranular material (Figs. 7, 8) will condense and constitute the viral core without previous existence of an internal viral envelope (Fig. 7) ;

2. after viral core constitution, in the electrondense material of inclusion there are differentiated membranes, that surround this viral core and will constitute the viral envelope (Fig. 9) ;

3. the virions are detached from the inclusion mass and released (Fig. 10). The external viral envelope is constituted by membranes which are *de novo* synthesized at the periphery of electrondense inclusion. The participation of Golgi apparatus as a membrane source to the external viral envelope formation of virions that are formed in inclusion is not proved.

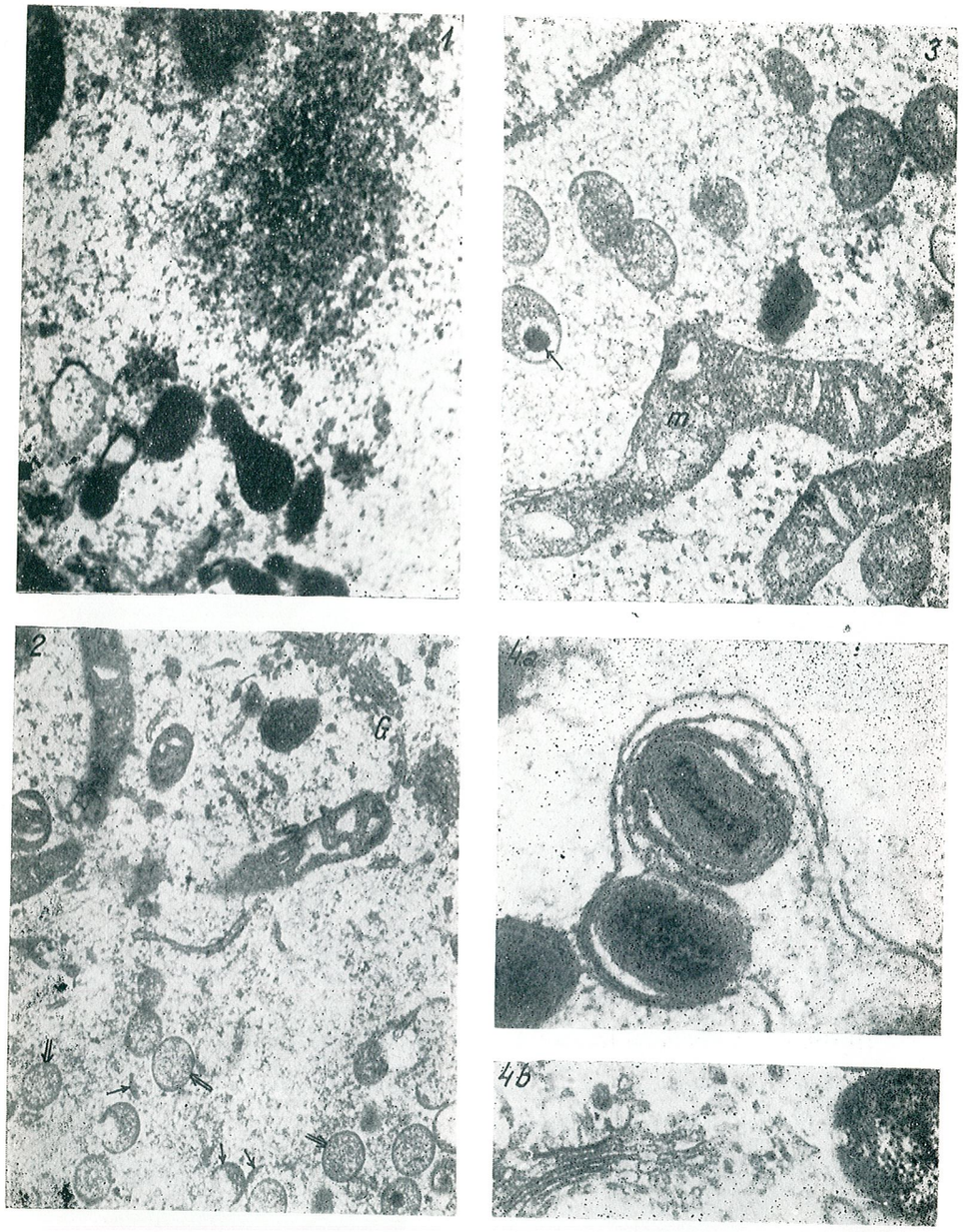
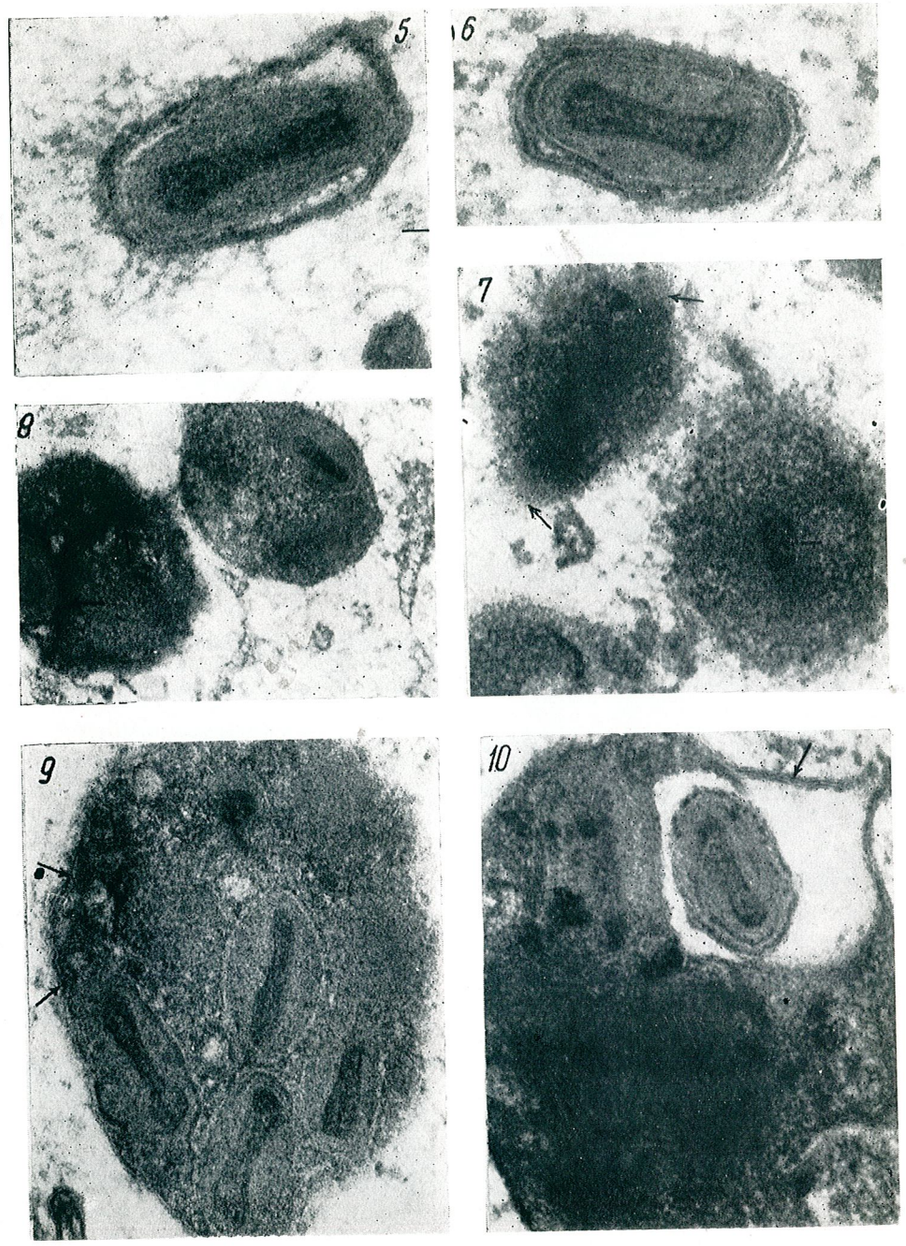


Fig. 1.—10 The cells of L_{23} cell line after 24 hours of vaccinia virus infection.
1, Viral factory. Electrondense formations are probably lysosomal bodies × 32.000. 2, The simple arrows point out the presence of membraneous calottes of viral origin. The double arrows point out the internal viral envelope constitution × 32.000. 3, The beginning of the viral core condensation process (arrow). Mitochondrial cristae (m) are enlarged × 46.000. 4, a) two virions surrounded by golgian cisternae × 157.000. b) the golgian apparatus in the same cell is hypertrophied × 46.000.



5, Golgi membrane condensation on the virion surface × 157.000. 6, Mature virion double layer covered × 157.000. 7, The arrows point out the uncondensed viral genome filaments. To the right viral core is built (arrow) × 115.000. 8, Two inclusion bodies ; to the left, arrows point out parallel filaments of viral genome × 70.000. 9, The virions in an inclusion. The inclusion periphery is membraneous (arrows) × 115.000. 10, The virion release from inclusion. Its external envelope is constituted by *de novo* synthesized membranes at the inclusion periphery × 115.000.

DISCUSSIONS AND CONCLUSIONS

As it is known [2], [7] the internal envelope of pox group virions is constituted by membranes *de novo* synthesized which gradually close the viroplasmic material. Morphological facts suggest that in the case of individual virions assembly, the internal envelope membrane is first differentiated initially appearing as reduced calottes ; these latter are gradually closed.

The chemical origin of these *de novo* formed membranes is a kind of glycoproteins that are electrophoretically distinct as regards their carbohydrate composition [7].

The role of hypertrophied Golgi apparatus in this viral morphogenesis step is probably to synthesize these glycoproteins, that will constitute the membrane of the virion envelope.

Later the Golgi apparatus participates directly by its cisternae to the process of external viral envelope formation but the direct continuity between these two structures have never been observed [4], [6], [8].

In the case of virion assembly in inclusion, the viral genome condensation takes place before the formation of the internal viral envelope which is *de novo* synthesized from the materials of the inclusion. In this situation, the participation of Golgi apparatus to the external viral envelope constitution is doubtful. The figures show that membranes are differentiated at the periphery of the inclusion, but they are structurally different from golgian cisternae. It can be stated that virion morphogenesis inside the inclusion takes place without direct participation of golgian membranes.

The morphological heterogeneity of external viral envelope origin might be a consequence of the existence of some antigenic differences between these two types of virions.

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SOIL NEMATODES OF THE BEECH FORESTS IN VLĂDEASA MOUNTAIN MASS

BY

IULIANA POPOVICI

The abundance, distribution and diversity of the soil nematode fauna in beech forests are discussed. The litter and the upper 10 cm of soil contained 67 to 96 per cent of the total nematode number. A significant decrease in the abundance and diversity of nematode populations with the increasing of soil depth is noted. The contribution of different ecological groups of nematodes to the formation of nematocenoses in different soil strata is illustrated.

The last International Colloquium on Soil Zoology assembled in Uppsala in 1976 — underlining the heterogeneous character of the soil fauna distribution in quite different habitats — also pointed out the great taxonomical and ecological diversity of biotic components, which coexist in the soil. It was emphasized the imperative of a more comprehensive study of biocenotic elements of the soil and, especially, the approach of functional aspects of these components. In this way it would be possible to use the biocenotic spectrum as an indicator of an ecosystem state.

The ecological studies of the last decade concerning the nematode fauna were directed on the structure, biomass and productivity of nematode communities, as well as on their impact on forest ecosystems. Few of these studies were devoted to soil nematode communities of beech forests, intensive studies — under stationary system — being undertaken in Denmark [3], [4] and England [2].

The present work aims to give estimates of the abundance and distribution of the nematode fauna and, also, to present aspects of the distribution of soil nematode ecological groups in the beech forests of Vlădeasa Mountain Massif (Western Carpathians).

STUDY AREAS AND METHODS

The altitudinal range of the beech forests (as. *Fagetum carpaticum)* in Vlădeasa Massif is comprised between 550 and 1080 m; these ecosystems are situated on acid brown soil, acid black soil, regosol and black rendzina. For the first three soil types humus form is forest mull and for the last one is calcaric mull type. The range of pH's is from 4.5 to 7.1; the organic content of the upper 10 cm of soil is 6.3 to 33.5 per cent, although in the soil down to the parent material it may be as low as 2.4 per cent.

Samples were collected (by taking 3×25 cc units) from each soil horizon during July-October 1977, trying to follow nematode distribution along the entire soil profile.

Both litter and soil were extracted using De Grisse's method [1] and the nematodes were fixed in TAF solution.

RESULTS AND DISCUSSION

Although our study concerns the distribution of nematode abundance along the entire soil profile of the beech forests, the present results are comparable with those obtained by Yeates [3], [4] for brown earth, and Phillipson et al. [2] for rendzina, for the top 6 cm of soil, depth at which their estimations were limited.

The most abundant nematode fauna — $3{,}490 \times 10^3/m^2$ — is noted in acid black soil followed by that of acid brown soil with $1{,}864 \times 10^3$ nematodes per square meter. The datum given by Yeates [3] for brown soil, in the top 6 cm of soil, was of $1{,}432 \times 10^3/m^2$.

The smallest nematode density noted by us — 494×10^3 — is sheltered in rendzina soil, the figure approaching very closely those indicated by Phillipson *et al.* [2] for the same type of soil ($386 \times 10^3/m^2$).

Figure 1 shows the distribution of nematode abundance in each soil stratum and the species abundance, too. The density of nematode populations in the litter and in the upper 10 cm of soil was found to be 67 per cent in acid black soil, 74 in regosol, 78 in acid brown soil, and 96 % in rendzina as per cent of the total number. Yeates [3] indicated 75 % in the top 6 cm, 80 % in 0—12 cm respectively in brown soil as % of total nematodes, while Phillipson *et al.* [2] noted a mean of 87 % in the top 6 cm of rendzina.

A significant decrease in the nematode abundance occurred in relation with the increasing of soil depth, so that the populations below 20 cm were about 10 % and at the level of parent material of the soil, the abundance represents only 2—7 % of the total nematode number.

We noted, also, differences in nematode abundance as concerning the soil type of the ecosystem; the first soil horizon of rendzina — mollic A horizon — sheltered 75 % of the total nematode fauna while the ochric A horizon of brown soil and the umbric A horizon of the black soil accounted only for 28 and 33 % respectively of the total nematode number.

The diversity of soil nematode fauna in beech forestes (Fig. 1) is remarkable. We noted changes in the degree of this diversity related with the soil type — its decrease from the rendzina and acid black soil to the brown one.

The distribution of nematode diversity related with the soil depth showed the concentration of the majority of species in litter and first soil stratum and a gradual reduction of species abundance with the increase of soil depth. These changes can be related with the abundance of food available for the nematode fauna and, also, with the changes in humidity and aeration degree of the soil (Fig. 1).

The remarkable abundance and species and ecological diversity of soil nematocenoses make necessary a comprehensive study of their contribution to the processes which took place in soil. Trophic spectrum of the nematode fauna appears to point out the state of soil and to define in this way the complex of habitat parameters which agreed with these biocenoses.

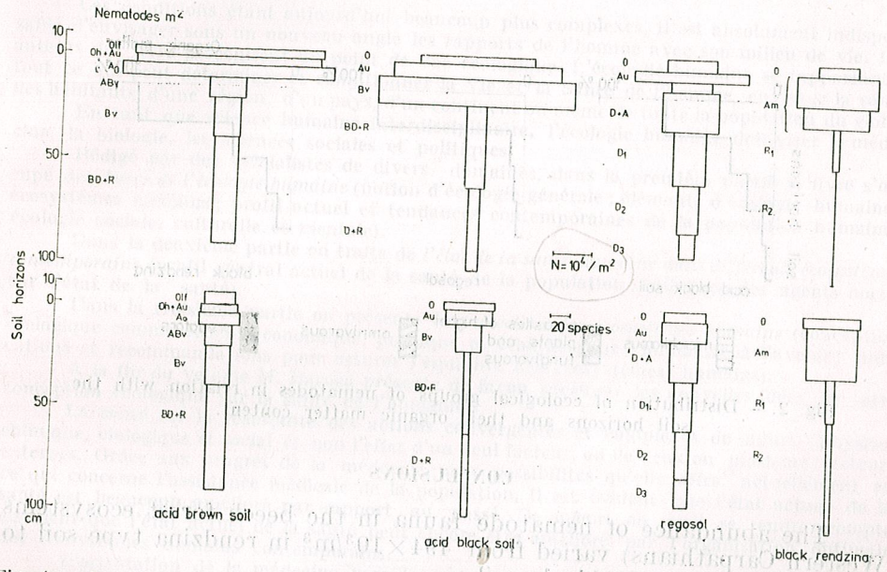

Fig. 1. — Distribution of abundance and diversity of nematode populations in the soil profile of the beech forests.

The participation of nematode ecological groups (trophic groups) to the formation of biocenotic communities of the beech forest soil indicated the prevailing of the plant parasitic group followed by a microbivorous one (Fig. 2). The distribution of ecological groups along the soil profile showed the concentration of microbivorous nematodes in the litter, correlated with the high organic content of this stratum (47—91 %). Litter accounted for 40 to 74 % of these nematodes while the soil strata contained only 7 to 32 % of them. Soil strata accounted for 33 to 83 % of the parasites of higher plants and fungivorous nematodes, and 5 to 30 % of omnivorous group. The predators showed the smallest densities up to 10 % of the total number.

Data emphasized the spreading of nematode populations in correlation to the trophic potential of the soil. The more profound study of these organisms becomes necessary taking into consideration their different functional spectrum.

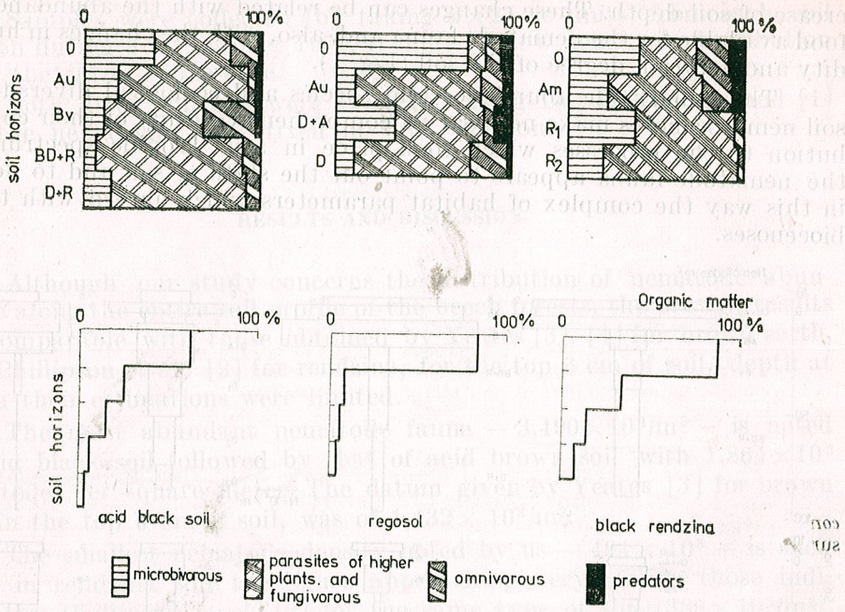

Fig. 2. — Distribution of ecological groups of nematodes in relation with the soil horizons and their organic matter content.

CONCLUSIONS

The abundance of nematode fauna in the beech forest ecosystems (Western Carpathians) varied from $494 \times 10^3/m^3$ in rendzina type soil to $3{,}490 \times 10^3/m^2$ in acid black soil.

The main mass of nematodes — 67 to 96 % of the total fauna — occurs in the litter and the upper 10 cm of soil. They were present down to the level of parent material but with a scarce abundance. A similar trend is noted for the distribution of nematode diversity.

Trophic structure of nematode communities showed the prevailing abundance in the order : plant parasitic nematodes > microbivorous > omnivorous > predators. The microbivorous species marked a preference for litter, while plant parasitic ones for mineral soil strata.

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M. BARNEA, A. CALCIU (rédacteurs), *Ecologie umană (Écologie humaine)*, I^er vol. 799 p., 125 figs., 76 tabl., Ed. medicală, București, 1979

L'ouvrage présente un intérêt particulier car il met en valeur le point de vue biologique concernant les problèmes de la santé humaine. L'hygiène classique s'est préoccupée en général des conditions de l'environnement, notamment de celles physico-chimiques nécessaires à assurer la santé de l'homme.

Ces conditions étant aujourd'hui beaucoup plus complexes, il est absolument indispensable d'envisager sous un nouveau angle les rapports de l'homme avec son milieu de vie. Les auteurs du livre préconisent un point de vue écologique. L'écologie humaine se rapportant à tout ce qui peut déterminer et conditionner la Vie et la Santé de l'homme envisage la santé des habitants d'une région, d'un pays, d'un continent ou même de toute la population du globe.

En tant que science humaine interdisciplinaire, l'écologie humaine doit viser la médecine, la biologie, les sciences sociales et politiques.

Rédigé par des spécialistes de divers domaines, dans la première partie le livre s'occupe des *bases de l'écologie humaine* (notion d'écologie générale ; éléments d'écologie humaine ; écosystèmes humains ; profil actuel et tendances contemporaines de la population humaine ; écologie sociale, culturelle et mentale).

Dans la deuxième partie on traite de *l'état de la santé humaine dans différents écosystèmes contemporains* (profil général actuel de la santé de la population ; influence des agents nocifs sur l'état de la santé).

Dans la troisième partie on présente *la protection des écosystèmes humains* (conception écologique comme repère économique, politique et éthique pour l'humanité à l'avenir ; indications et recommandations pour assurer l'équilibre des écosystèmes humains).

A la fin du volume M. Barnea présente de façon succincte les nouvelles idées de cette conception écologique sur la santé de l'humanité.

La santé est la résultante des actions convergentes et complexes du milieu physico-chimique, biologique et social et non l'effet d'un seul facteur, ou de deux ou plusieurs facteurs externes. Grâce aux progrès de la médecine, aux possibilités qu'elle offre actuellement en ce qui concerne l'assistance médicale de la population, il est évident que l'état actuel de la santé est beaucoup amélioré par rapport au passé. De même, on peut se rendre compte aisément que l'état actuel de la santé peut encore être amélioré par l'organisation optimale des écosystèmes humains contemporains.

L'orientation de la médecine vers les principes écologiques peut être un guide pour l'amélioration du rapport : homme — environnement. Le respect des lois de la protection de l'environnement constitue le premier pas ver s l'équilibre qu'on souhaite établir entre l'homme et son milieu. Dans cette action, il faut aussi changer certains rapports de l'homme avec la nature et surtout avec les milieux nocifs, conséquence du développement impétueux de l'industrie. Des efforts doivent être faits pour réaliser une écosphère saine pour l'homme actuel, ce qui se traduit par le maintien d'un équilibre, absolument nécessaire, entre l'homme et son milieu complexe, afin de rendre meilleure la santé de la population.

Par les thèmes abordés, cet ouvrage constitue une précieuse contribution à la solution de l'un des problèmes les plus actuels du monde moderne.

Le livre ouvre une perspective nouvelle aux rapports qui doivent exister de nos jours entre l'homme et son environnement.

E. A. Pora

H. ZAMFIR, *Efectele unor poluanți și prevenirea lor (Effets de certains polluants et leur prévention)*, I^er vol. 176 p., 44 figs, 60 tabl. de données, Ed. Academiei, București, 1979

C'est grâce aux sciences que l'industrie a pu fournir à l'homme tout ce dont il a besoin ; c'est grâce aux sciences aussi que l'homme est arrivé à connaître les effets des polluants industriels et à prendre de mesures pour les prévenir. C'est là l'idée centrale de ce livre extrême

ment bien venu, car il donne des résultats des investigations effectuées par l'auteur dans les zones polluées de Roumanie. Pour chaque cas étudié il recommande des mesures efficientes pour combattre ou pour limiter les effets négatifs sur l'environnement et sur la santé de l'homme.

Le livre s'occupe successivement des polluants naturels, physiques, chimiques et biologiques. On expose ensuite les résultats qui peuvent mener à la protection des sources aquatiques, des lacs de barrage, des espaces verts et de la normalisation des substances chimiques et cancérigènes.

Ce volume fournit une riche quantité d'informations sur la pollution à tout ceux préoccupés de la santé de l'homme, qui peuvent trouver ici des données sur le grade de pollution et les mesures de protection qui s'imposent.

A la fin du livre l'auteur aborde, dans un chapitre spécial, la possibilité d'une recherche interdisciplinaire sur les problèmes de la pollution. Il est ainsi d'accord avec le professeur Maurice Fontaine, qui créa en 1973 le terme de « *molysmologie* » pour une discipline synthétique, qui pourra s'occuper de tous les aspects de la pollution : production des polluants, action biologique et médicale, moyen de les combattre, etc. Une telle discipline devrait avoir un institut spécial car le problème de la pollution est un de ceux qui influencent aujourd'hui toute l'activité de l'humanité.

Le livre plaide pour la nécessité actuelle des investigations interdisciplinaires, afin que l'on puisse arriver le plus tôt possible à des solutions dans le problème si important de la santé et de l'existence pour assurer à l'homme un milieu sain et en plein accord avec ses besoins.

E. A. Pora

P. G. PLOAIE, ZOE PETRE, *Introducere în microscopia electronică cu aplicații la biologia celulară și moleculară (Introduction in electron microscopy with application to cellular and molecular biology)* (preface by Radu Codreanu), Ed. Academiei, București, 1979, 330 pp., 112 figs., 930 refs.

The work is an excellent guide especially for the young researches in the field of molecular and cellular biology, describing a wide range of methods checked and applied by the authors in the field of ultrastructure during a period of more than 15 years.

In the first chapter "Electron microscopy and the field of investigation at ultrastructural and molecular level", the readers are informed about the role of electron microscopy in obtaining structural information in cellular and molecular biology, concerning the levels of the ultrastructural organization available to the electron microscopy, as well as the possibilities offered by this technique.

In the next chapter "The electron microscope : history, construction and principles of functioning" the authors present the principles underlying the construction and functioning of different types of electron microscopes existent all over the world. In addition, the authors comment the conditions necessary for obtaining optimal performance and high resolution electron-microscopic images.

The second part begins with a large chapter (3) entitled : "The preparation of biological materials for electron microscopy investigations", where the authors present a detailed description of all the phases involved in the preparation of the biological material for the electron microscopic study. An important consideration is given to different variants and work procedures as applied to each specific phase, the necessary materials, the most indicated recipes, apparatuses and work auxiliaries, practical recommendations.

In chapter, 4, "Location of some molecular components and biosynthesis processes at cellular level", methodological and technical procedures the study ot biosynthesis processes are presented. Thus, the researcher is acquainted with application of various techniques such as : autoradiography which uses radioactive precursors, the *in vitro* investigation of the enzymic equipment and the blocking of different steps in the biosynthesis processes. Following up such techniques, the interested reader is able to identify and localize on his/her biological specimens certain steps of cell biosynthesis, different enzymic activities, antibodies, polysaccharides, as well as tracers of protein transport and microanalysis ot certain chemical elements.

Chapter, 5, "Cell fractioning and visualization of cell organelles and biomolecules", describes the procedures for cells isolation, their fractionation and purification of cellular components, viruses, bacteriophages and nucleic acids in order to make them accessible for the electron microscope study.

In the last chapter, "Organization of the electron microscopy laboratory and the protection against radiations", the authors give useful recommendations for newly built laboratories and their personnel.

One can conclude that the authors have succeeded in presenting in a concentrated form a full range of methods and techniques currently used today. A practical-methodological exposition, the consultation of a large bibliographical information (930 refs.) as well as the comments on each method based on the author's own experience, make the book very valuable.

The work is recommended to all the specialists working in the field of biology, medicine and agriculture interested in studying the living matter at the cellular and molecular level.

Constantin Crăciun

AVIS AUX AUTEURS

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

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

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

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

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