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SOMMAIRE

LEVIAPSEUDES LONGISPINA SP. N. DE LA PLAINE ABYSSALE DEMERARA DE L'AMAZONE

PAR

MIHAI BĂCESCU

The study of part of the September 1980 material collected in the French Guinea and the North of the cone of the Amazon by the Demeraby campaign organized for the exploration of the Demerara abyssal plain by the COB (Oceanological Center of Brittany) and the Paris National Museum of Natural History allowed the identification of a new species of *Leviapseudes* — *L. longispina* (4400 m).

Diagnose. Tanaidacé petit (environ 10 mm ♂ ♀) à pléopodes puissants (♂ ♀) et garnis d'épines hyposphéniales énormes, tout aussi longs que la hauteur des segments qui les supportent. Chélipèdes extrêmement fins. Organe pénial cylindrique, avec une épine énorme courbée antérieurement.

La griffe du pléopode V ♂ lisse ; celle de la ♀, à 3—4 denticulations, dont la distale juste près de son bout.

Dans 3—4 stations de la campagne Demeraby, organisée par le Centre Océanologique de Bretagne en collaboration avec le Muséum National d'Histoire Naturelle-Paris, à l'Est de la Guyane française et au Nord du cône de l'Amazone en septembre 1980, nous avons dépisté un *Leviapseudes* à part. — *L. longispina* — que nous décrivons plus bas.

MATÉRIEL

— CP05, 8°08′22″N ; 44°02′20″0 ; 4430 m : 1 ♂ ad. et 1/2 ♂ ad., 1♀M (chalut).
— KG08, 10°25′13″N/46°45′93″0, 4850 m ; 1♂ ad.
— KG07, 8°08′45″N ; 49°04′12″ (Carottier) 4440 m.

Description du mâle (Fig. 1)

Tégument pauvre en phanères, faiblement calcifié chez tous les 5 exemplaires et les restes d'exemplaires étudiées ; presque transparent, hyalin, raison pour laquelle on distingue difficilement les reliefs du céphalothorax.

La carapace est pourvue d'un long rostre pointu, sans dilatations à la base, ses bords passant directement vers la ligne de séparation des lobes oculaires. Les apophyses postoculaires grandes, larges (Fig. 1 B). L'épistome présente une épine longue (Fig. 1I).

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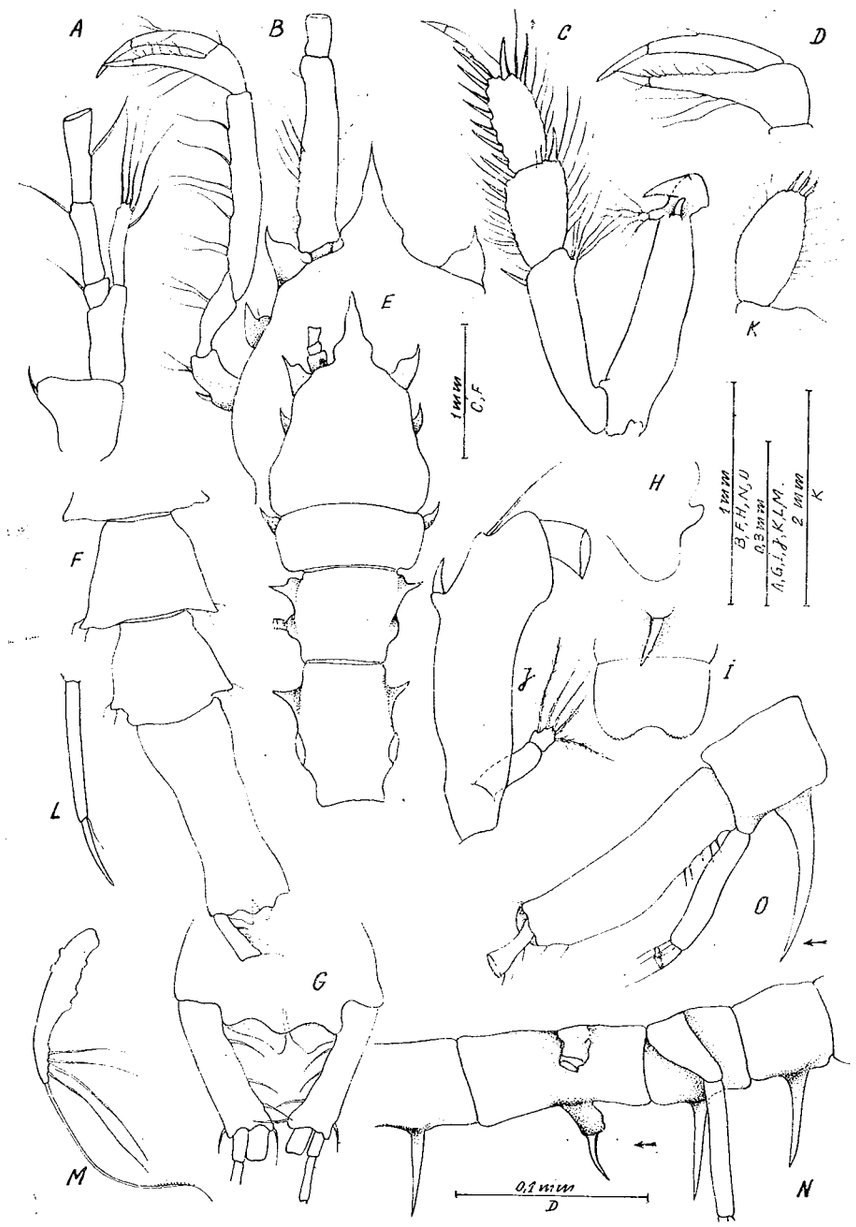

Fig. 1. — *Leviapseudes longispina* ♂.

A, A_1 ; B partie antéro-laterale de la carapace, avec le chelipède gauche ; C, péréiopode II ; D, chéla, grossie ; E, céphalothorax d'une autre♂ ; F, partie terminale du pléon ; G, extrémité du pléotelson ; H, Bouclier latéral de la carapace ; I, labrum et l'épine épistomale ; J, ischiopodite du I^{er} péréiopode ; K, lobe du labium ; L, dactylogriffe du V^{e} péréiopod ; M, article terminal du palpe de la maxillule ; N, partie terminale du thorax et le premier segment du pléon de profil ; la flèche, l'organe pénial ; 0, pléotelson et le dernier pléonite avec son long hyposphaenium.

Des apophyses coxales pointues et des apophyses épineuses à peu près pareilles, dans la partie antérieure, élargie, du péréionite libre II (Fig. 1 E). La forme et les bords des autres péréionites peuvent être vus dans les figures 1 E et 2 E. Le bouclier latéral de la carapace avec la marge inférieure lisse (Fig. 1M.). Les pléonites trapézoides, un peu plus longs que leur largeur au niveau des coins postérieurs ; les épimères avec seulement 2 soies (Fig. 1 F).

Sous tous les segments du corps, chez le ♂, d'énormes épines hyposphéniales pointues, au moins tout aussi longues que la hauteur des segments qui les supportent ; ceux au-dessous des pléonites sont seulement un peu plus courts que les bases des pléopodes (Fig. 1 O). L'organe pénial conique, avec une immense épine flagelliforme courbée antérieurement (Fig. 1 N, la flèche).

Le pléotelson court et épais, un peu plus long que les deux derniers pléonites et un peu plus large que haut (comparer Fig. 1 O avec 2 A) ; il a une légère enflure inférieure vers le milieu de sa longueur ; une série de poils-épines inférieurs dans la moitié antérieure ; une petite touffe de poils sur la proéminence dorsale apicale et 2 poils-épines inférieurs. Rapport longueur hauteur : 3,8.

L'antennule avec l'article basal à 2 groupes de phanères externes : l'un formé de petits poils proximaux, l'autre, d'une sète longue et de 4 poils sensitifs implantés au milieu de cet article ; du côté interne, seulement 3 soies (Fig. 1 A).

Six articles au petit flagelle et 23 au grand, chacun avec un bouquet d'esthétasques très fines ; seulement une-deux plus épaisses sous-terminales (sur le 4^{e} et le 3^{e} article distal). Le long flagelle est bien plus épais que celui court et il semble poilu à cause de la multitude d'esthétasques fines. L'antennule a un article proximal avec un prolongement interne proéminent qui finit par un poil. L'écaille a 6 sètes plumées (Fig. 1 A) et l'endopodite a 9 articles, quelques-uns avec des sètes longues, plumées.

Le labrum (Fig. 1 I) avec l'excavation lisse et sans garniture de poils latéraux. Le labium aux lobes finissant par 3 petites épines fidées (Fig. 1 K).

La mandibule à palpe court triarticulé ; son article basal présente 2 sètes et un petit tubercule. Pars masticatoria comme une langue molle ; pars incisiva avec des dents non chitinisées.

Le palpe biarticulé de la maxillule a l'article terminal court, en quelque sorte gaufré avec seulement 6 phanères pourvus d'ardillons (Fig. 1 M). Le maxillipède d'une morphologie commune au genre, à 3 rétinacles symétriques.

Chélipèdes longs et fins ; ischium armé d'une épine puissante dans le tiers antérieur (Fig. 1 J), suivie de trois épines-sètes minuscules et d'une sète longue sous-terminale.

Pince extrêmement fine, avec de longues griffes lamellaires, celle du dactyle dépassant visiblement celle du propode (Fig. 1 D). Exopodite à 4 sètes simples (Fig. 1 J).

Le péréiopode II a une apophyse coxale épineuse et une épine terminale sur l'ischium ; pour la forme des articles et leur phanerotaxie voir figure 1 C ; l'exopodite toujours avec 4 sètes.

Le propode (Fig. 1 C), armé de 7 épines inférieures qui ont seulement 2 sètes simples entre elles, a une longue dactylogriffe.

Le reste des péréiopodes d'une extrême finesse. Le péréiopode V plus petit, avec une dactylogriffe fine, sans dilatation proximale et sans denticulations sur la griffe (Fig. 1 L).

Les pléopodes parfaitement identiques à ceux de la femelle (Fig. 2 B), avec un sympode puissant, nettement plus long que les rames ; celles-ci sont larges, presqu'égales.

Les uropodes avec insertion sous-terminale présentent 4 poils spiniformes internes et un poil sous-terminal externe (Fig. 1 G) ; le petit flagelle avec 7 articles ; le grand absent.

Taille : 10 mm.

Description de la femelle (Fig. 2)

Le rostre, les lobes postoculaires, les pièces buccales, le nombre d'articles des flagelles de l'antennule et de l'antenne, le chélipède et la forme des thoracomères d'une ressemblance frappante avec les pièces respectives du mâle.

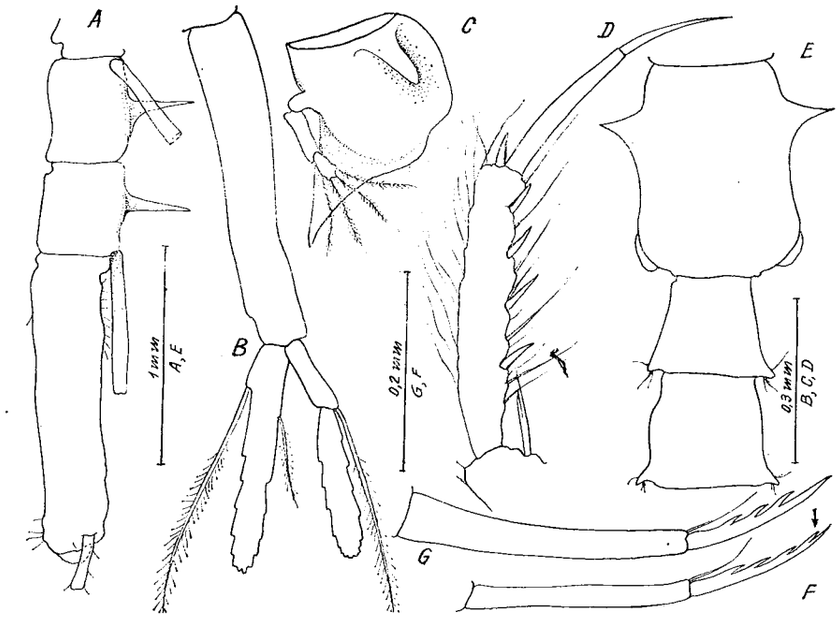

Fig. 2. — *Leviapseudes longispina* ♀.

A, l'extrémité du pléon, de profil, B, pléopode I ; C, exopodite et l'apophyse coxale de péréiopode II ; D, propode et dactylogriffe du III^{e} péréiopode ; E, VII^{e} segments thoracique et les deux premiers pléonites ; F, dactylogriffe du V^{e} péréiopode en comparaison de la dactylogriffe V du *Leviapseudes longissimus* (Fig. II, G).

L'A_2 a 9 articles à l'endopodite et 5 sétae à l'écaille. La dactylogriffe de la chéla dépasse beaucoup celle du propode.

Seul le pléotelson est plus long (tout aussi long que 3 pléonites pris ensemble) et plus mince (Fig. 2 A) ; le rapport longueur/hauteur est : 3 : 1 ; les pléonites sont toujours trapézoides, un peu plus longs que chez le ♂ (Fig. 2 E).

Le péréiopode II a aussi 7 épines, la marge interne du propode presqu'ovale. Le péreiopode III comme dans la figure 2 D et l'exopodite comme dans la figure 2 C.

La griffe fine du péréiopode V (rapport longueur : largeur à la base = = 10,6) présente 4—5 denticules courts (chez les adultes), celui distal étant presque sous-terminal (la flèche, Fig. 2 F) ; par ce détail morphologique, *L. longispina* se distingue de tous les autres membres du genre et particulièrement de *L. longissimus* qui a lui aussi la dactylogriffe V très fine, mais avec le rapport 7, pour la griffe (Fig. 1 G). La dactylogriffe ne présente pas de trace d'enflure à la base ; les pléopodes absolument identiques à ceux du mâle (Fig. 2 B).

L'exopode de l'uropode a 7 articles, l'endopodite en a 30.

Taille : 10 mm environ.

Derivatio nominis. Le nom spécifique est suggéré par la longueur inhabituelle des épines hyposphéniales, particulièrement de celles sous-abdominales.

Holotype : ♀ M, St. CP05 Demeraby, déposé au Muséum d'Histoire naturelle de Paris, au Laboratore de Crustacés, de même que 2 paratypes.

Allotype ♂, sous le no. 683, au Muséum « Grigore Antipa » et 2 paratypes ♀♂ sous le no. 684.

OBSERVATIONS

La présence des 5 paires de pléopodes chez les ♀♀ développés tout aussi bien que chez le ♂ indique un mode de vie similaire chez les deux sexes, une vie probablement benthonéctonique, sans grands efforts de nager, compte tenu du faible développement de la musculature des pléonites, par contraste à *L'hanseni*, par exemple.

L'espèce est facile à reconnaître par les épines sternales d'une longueur exceptionnelle, par le manque d'un dimorphisme aux pléonites, aux pléopodes et péréionites. C'est une espèce abyssale qui vit sur un fond vaseux de 4000 à 5000 m, en compagnie de *Sphyrapus malleolus* M. Sars et de *Leptoapseudes gracillimus* N.& St.

Reçu le 20 octobre 1982

Musée d'histoire naturelle « Grigore Antipa »
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IDÉES NOUVELLES DANS LA MORPHOLOGIE, LA PHYLOGÉNIE ET LA CLASSIFICATION DES LÉPIDOPTÈRES

PAR

EUGEN V. NICULESCU

It is emphasized that in the Lepidoptera, only the study of the exoskeleton can provide real data in order to establish the true phylogenetical relations and to elaborate a natural phylogenetical classification, as well as to build a suitable taxonomy at all levels, from the species to the suborder.
The classification proposed until the present are not strictly phylogenetic, because they do not rely on the exoskeleton, but on the size of the body, the mandibles of the chrysalides, the mouth-parts of the adults, the female genital orifice, the shape of the abdominal legs of the larvae, etc. None of the modern classifications, except the one based on the exoskeleton, can be accepted.

La littérature lépidoptérologique regorge de classifications diverses que nous avons analysées et critiquées dans plusieurs travaux [5], [8 — 11]. Les caractères morphologiques utilisés jusqu'à présent concernent la trompe, les pattes, la nervation, le couplage des ailes, l'orifice génital ♀, les mandibules de certaines chrysalides, les pièces buccales atrophiées de certaines espèces *(imagines)*, la taille naine du corps (chez les Nepticulidae), les pedes spurii et les organes tympanaux.

Mais personne ne s'est penché, jusqu'à présent, sur l'exosquelette à des fins taxonomiques et phylogénétiques et c'est pourquoi la classification des Lépidoptères reste encore « primitive », c'est-à-dire ne reflète pas les véritables relations phylogénétiques dans tous les taxa.

Des recherches de morphologie théorique sur l'exosquelette, ont été publiées par K. Jordan [2], N. Kusnetzov [4], Shepard [16], Cl. Lemaire, etc.; mais personne n'a fait paraître d'études de morphologie « appliquée » — sous l'aspect taxonomique et phylogénétique, ce que nous avons dénommé « morphologie taxonomique » [12]. Nos recherches de morphologie taxonomique sur l'exosquelette se sont avérées très fructueuses et nous ont fourni un riche matériel comparatif, ce qui nous a permis d'élaborer une nouvelle classification générale des Lépidoptères et, à l'intérieur de la superfamille des Papilionoidea d'établir un grand nombre de nouvelles classifications des familles Pieridae, Nymphalidae, Satyridae [14] et Papilionidae (sous-presse).

L'exosquelette est formé d'un grand nombre de sclérites, mais tous n'étaient connus jusqu'à présent. Nous avons entrepris des recherches plus approfondies que nos prédécesseurs et avons découvert ou nommé 25 sclérites nouveaux, surtout dans le mésothorax.

En examinant le thorax chez plus de 2000 espèces appartenant à presque toutes les familles du monde, nous avons constaté que le sclérite nommé par K. Jordan [2] parasternum (anepisternum) existe chez tous

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les papillons nocturnes et manque chez tous les Rhopalocères. Ainsi nous avons avancé l'idée, nouvelle, que les Lépidoptères se sont développés suivant deux lignées phylètiques : l'une représentée par les papillons dotés d'un parasternum, l'autre, dépourvus de parasternum. D'où l'idée de diviser l'ordre en deux sous-ordres : Parasternia et Aparasternia. Cette idée a été reçue avec chaleur par certains lépidoptéristes. Ainsi M. Jean Bourgogne nous a écrit en 1968 : « Vous avez réalisé là un travail très important. Vos recherches sur le squelette sont très intéressantes et donnent un caractère nouveau utilisable en systématique. Il est intéressant d'y voir un rapprochement entre les Papilionidea et les Hespérides, si différents par ailleurs. L'intérêt de votre découverte est de préciser l'isolement des Papilionoidea par rapport aux autres Lépidoptères et de suggérer que les Hespérides seraient sur la voie des Papilionoidea ».

Chacun des deux sous-ordres établis par nous présente aussi d'autres caractères que nous définissons, pour plus de clarté, dans le tableau synoptique ci-dessous.

Caractères	Aparasternia	Parasternia
1. Parasternum	Absent (présent seulement chez les Hesperiidae)	Présent
2. Pseudoparasternum	Présent ou absent	Absent
3. Protomerum	Absent	Présent, rarement absent
4. Pseudoprotomerum	Présent ou absent	Absent
5. Mesopons	Sclérite très allongé antéro-postérieurement, fort sclérifié	Sclérite triangulaire large, le plus souvent faiblement sclérifié
6. Mesophragma	Munie dorsalement de deux processus sclérifiés	Dépourvue de processus dorsaux
7. Suture précoxale	Absente ou présente	Présente
8. Le couplage des ailes	Du type amplexiforme rarement frénate	Du type jugate, jugofrénate ou frénate, rarement du type amplexiforme
9. Vol	Diurne	Nocturne, rarement diurne
10. Le premier tergite abdominal	Culcitiforme	Diversement conformé, jamais culcitiforme

Il résulte de ce tableau que les deux lignées phylétiques — et donc les deux sous-ordres — sont bien séparés. Un examen attentif du tableau nous montre encore que l'évolution de l'ordre Lepidoptera s'est produite dans le sens d'une réduction du nombre des sclérites et des sutures. Les Parasternia se révèlent les plus primitifs, offrant le plus grand nombre de sclérites et sutures, les Aparasternia sont les plus évolués, possédant moins de sclérites et sutures. Certains sclérites sont « exclusifs », existant ou manquant uniquement chez l'un ou l'autre des deux sous-ordres. Ainsi, le parasternum, le protomerum le mesopons (large et faiblement sclérifié) et la mesophragma (dépourvue de processus dorsaux) se rencontrent chez les Parasternia seulement ; au contraire, le pseudoparasternum, le pseudo-

protomerum, le praemesepimerum, le mesopons (étroit, allongé et fortement sclérifié) et la mesophragma (pourvue de processus dorsaux) ne s'observent que chez les seuls Aparasternia. Mentionnons enfin que certains sclérites et sutures sont en voie de disparition comme par exemple, le pseudoparasternum et le pseudoprotomerum (tous deux absents chez les Pieridae), le praemesepimerum, la suture précoxale (absente chez les Pieridae), la suture du mesomerum. Du fait de la disparition de la suture précoxale tout l'espace situé entre le discrimen et le paramerum est occupé par un seul sclérite, structure existant seulement chez les Pieridae, Papilioninae et Parnassinae (sous presse). Chez tous les autres Lépidoptères, cet espace est divisé en deux sclérites (mesosternum et mesepisternum) par la suture précoxale.

Nos investigations de morphologie taxonomique nous ont permis de mettre en lumière de nouvelles relations phylogénétiques, différentes de celles dégagées antérieurement par d'autres chercheurs. Ainsi, nous avons constaté que les Papilionoidea n'offrent pas de relations phylogénétiques avec les Pterophoroidea comme le suggère la classification de W. Forster [1] qui, en suivant S. G. Kiriakoff, a intercalé les Papilionoidea entre les Pterophoroidea et les Bombycoidea. L'erreur de Kiriakoff provient du fait qu'il réunit dans un même groupe (Harmoncopoda) les trois superfamilles en se basant sur la structure, ressemblante, des pedes spurii semicoronati. Nous avons déjà combattu cette erreur et n'y revenons plus [9]. Les Papilionoidea présentent des relations de proche parenté avec les Hesperioidea et c'est pourquoi nous avons réuni ces deux superfamilles dans le sous-ordre Aparasternia [7]. Pour établir des divisions à ce niveau il faut utiliser l'exosquelette et non pas les pedes-spurii.

C'est toujours l'exosquelette qui nous a montré que les Pieridae ne constituent pas les plus primitifs des Papilionoidea comme l'admet Al. Klots [3], mais, tout au contraire, s'avèrent les plus évolués, vu leurs relations de proche parenté avec les Papilionidae. Dans sa classification, W. Forster cite les familles composant la superfamille Papilionoidea dans l'ordre de présentation suivant : Papilionidae, Pieridae, Danaidae, Morphidae, Brassolidae, Satyridae, Nymphalidae, Libytheidae, Riodinidae et Lycaenidae. Selon Forster les Papilionidae sont les plus primitifs et les Lycaenidae les plus évolués. Nous avons agencé ces familles dans un tout autre ordre : Riodinidae (les plus primitifs), Lycaenidae, Satyridae (y compris les Brassolidae), Libytheidae, Nymphalidae (y compris les Morphidae), Papilionidae et Pieridae (les plus évolués). L'exosquelette nous renseigne aussi sur le degré évolué (ou primitif) des sous-familles et des genres. Parmi les Pieridae, les plus évolués sont les Dismorphiinae et les plus primitifs, les Coliadinae ; chez les Nymphalidae les plus primitifs sont les Charaxinae et chez les Papilionidae ce sont les Zerynthiinae. Enfin, pour le groupe — « genre » nous n'exposons ici qu'un seul cas : celui du genre Archon [15], transféré par nous de la sous-famille des Parnassiinae dans la sous-famille des Zerynthiinae, plus primitive que celle des Parnassinae, elle-même plus primitive que celle des Papilioninae.

Dans les classifications récentes, les Noctuoidea sont placés à l'extrême opposé des Micropterygoidea, considérés comme les Lépidoptères les plus évolués. Nous n'acceptons pas cette conception puisque l'exosquelette la dément. Le nombre des sclérites et des sutures chez les Noc-

tuoidea est plus grand que chez les Papilionoidea; ils possèdent un parasternum, un protomerum et le mesopons et la mesophragma du type Parasternia. C'est pourquoi nous ne pouvons partager cette opinion et considérons que les plus évolués des Lépidoptères sont les Papilionoidea et non pas les Noctuoidea [6].

Après définition des relations phylogénétiques en fonction de l'exosquelette, l'élaboration de la classification du groupe est chose facile. Le tout consiste dans le choix des caractères, car n'importe quel caractère morphologique n'exprime pas les véritables relations phylogénétiques. Ainsi pour décider si un papilionide appartient ou non au genre Eurytides, il est pertinent d'examiner l'uncus et le superuncus et non pas la harpe; dans ce genre la harpe fournit de bons caractères spécifiques, l'uncus et le superuncus, de bons caractères génériques. Si nous voulons être fixés quant à l'appartenance d'un papillon au sous-ordre Apparasternia, il faut examiner le mesopons et la mesophragma, mais s'il s'agit de connaître la famille à laquelle il appartient il s'indique d'examiner le proscutellum et le mesoclidium. Le choix fait par Kiriakoff pour établir certains taxa en utilisant les mandibules des chrysalides, la taille naine du corps, les pedes-spurii, etc. a été mal inspiré comme nous l'avons montré dans un travail antérieur [9]. Ces caractères ne sont pas équivalents morphologiquement et n'ont pas par conséquent de valeur phylogénétique, quoique l'auteur se considère comme un «phylogénéticien conséquent»! Nous avons montré qu'à un niveau taxonomique donné, tous les taxa doivent être établis par des caractères équivalents morphologiquement et non pas selon des caractères morphologiques disparates, comme dans la classification de Kiriakoff. Nous avons choisi, au niveau sous-ordinal la présence (ou l'absence) du parasternum et du protomerum ainsi que la forme différente de deux sclérites (mesopons et mesophragma), chacun de ceux-ci ayant une forme très constante dans les deux sous-ordres. Ces caractères reflètent vraiment le développement de l'ordre Lépidoptère selon deux lignées phylétiques et dans chacune de celles-ci les papillons offrent des relations de parenté soit plus proches soit plus éloignées, non pas en fonction de la taille du corps ou de la structure des pedes-spurii — soumis aux convergences mais bien suivant la conformation des sclérites de l'exosquelette et de l'armature génitale.

Nous sommes convaincus que l'avenir de la Lépidoptérologie appartiendra à l'exosquelette qui seul aboutira à la révolution taxonomique tant désirée et attendue par la plupart des lépidoptéristes. Le moment est venu d'en finir avec les fantaisies et les «innovations catastrophiques»[1] si nous voulons élaborer une classification réelle, phylogénétique.

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THE PARASITE COMPLEX OF GYPSY MOTH *(LYMANTRIA DISPAR* L.) *(LEP.: LYMANTRIIDAE)* IN THE OAK WOODS FROM SOUTHERN ROMANIA

BY

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The parasites of larval and pupal *Lymantria dispar* and their efficiency in seven oak woods from the Giurgiu county, in southern Romania are established. This parasite complex includes 18 species: 1 *Braconidae* (Hym.): *Apanteles* sp.; 3 *Ichneumonidae* (Hym.): *Euceros superbus* Kriechb., *Theronia atalantae* (Poda) and *Coccygomimus instigator* (F.); 1 *Chalcidoidea* (Hym.): *Brachymeria intermedia* (Ness); 1 *Torymidae* (Hym.): *Monodontomerus aereus* Walk.; 1 *Eulophidae* (Hym.): *Eulophus larvarum* L.; 6 *Tachinidae* (Dipt.): *Exorista larvarum* (L.), *Compsilura concinnata* (Meig.), *Carcelia separata* (Rond.), *Blondelia nigripes* (Fall.), *Kramerea schuetzei* Kram. and *Phrixe prima* B.B.M.; 2 *Muscidae* (Dipt.): *Muscina stabulans* Fall. and *Muscina pabulorum* Fall.; 2 *Sarcophagidae* (Dipt.): *Parasarcophaga uliginosa* Kram. and *Pseudosarcophaga affinis* Fall. and 1 *Mermithidae* (Nematoda): *Hexamermis albicans* (Siebold). The species *Euceros superbus, Parasarcophaga uliginosa* and *Phryxe prima* were obtained, for the first time in science, from *Lymantria dispar*. The highest parasitization degree of larval and pupal gypsy moth in this area was given by Diptera.

The gypsy moth *(Lymantria dispar* L.) is the main defoliator of the oak woods in Romania, with repeated outbreaks and causing heavy defoliations, especially in the south of the country [3]. These defoliator populations are limited by a parasite complex which can often reach a high degree of parasitism.

This paper deals with the larval and pupal gypsy moth parasite complex and its contribution to the limitation of the host populations.

MATERIALS AND METHODS

Between 1977 and 1980 we collected larvae and pupae of *L. dispar* from seven oak woods in the Giurgiu county: Dăiţa, Frasinu, Băneasa, Arbori, Nebuna, Rușii lui Asan and Bălăşcuţa, in order to obtain the natural enemies of this pest. These woods contain *Quercus cerris* L., *Q. frainetto* Ten. and *Q. pubescens* Wild.

During this period there were collected 9496 larvae and 5670 pupae of *L. dispar*, which were examined in the laboratory in order to obtain the parasites and to establish the degree of parasitism. The larvae in their third to sixth stage were collected in late May and early June, while the pupae were collected between June 15 and August 10.

Each larva and pupa was put in a vial of 20 cm^3, corked with cotton-wool and kept in the laboratory at room temperature, avoiding dryness. The parasite hatching was watched daily.

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RESULTS AND DISCUSSIONS

Of the total of 15,266 collected larvae and pupae of *L. dispar*, 2624 have been parasitized, which represents a total parasitization index of 17.19% (3.32% for larvae and 13.87% for pupae). This parasitization index was achieved by the convergent action of 17 species of the entomophagous parasite insects and one species of parasite nematodes (Table 1).

Table 1

Identified Larval and Pupal Parasites of Gypsy Moth

Braconidae	*Apanteles* sp.
Ichneumonidae	*Buceros superbus* Kriechb.
	Theronia atalantae (Poda)
	Coccygomimus instigator (F.)
Chalcididae	*Brachymeria intermedia* (Nees)
Torymidae	*Monodontomerus aereus* Walk.
Eulophidae	*Eulophus larvarum* L.
Tachinidae	*Exorista larvarum* L.
	Compsilura concinnata (Meig.)
	Carcelia separata (Rond.)
	Blondelia nigripes (Fall.)
	Kramerea schuetzei Kram.
	Phryxe prima B.B.M.
Muscidae	*Muscina stabulans* Fall.
	Muscina pabulorum Fall.
Sarcophagidae	*Pseudosarcophaga affinis* Fall.
	Parasarcophaga uliginosa Kram.
Mermithidae (Nematoda)	*Hexamermis albicans* (Siebold)

It can be seen that of the 18 species of natural enemies, 5 species are both larval and pupal parasites: *E. superbus*, *Exorista larvarum*, *C. concinnata*, *P. affinis* and *K. schuetzei*; 6 species are only larval parasites: *Apanteles* sp., *Eulophus larvarum*, *P. prima*, *C. separata*, *B. nigripes* and *H. albicans* and 7 species are only pupal parasites: *T. atalantae*, *C. instigator*, *B. intermedia*, *M. aereus*, *M. stabulans*, *M. pabulorum* and *P. uliginosa* (Fig. 1).

Exorista larvarum, *C. concinnata* and *B. intermedia* are the most effective parasites in the limitation of the host populations, with 67.38% from the total parasitism. The first two species have 63.41% of the total parasitism. *C. concinnata* is more frequent, as larval parasite, while *Exorista larvarum* as pupal parasite.

The contribution of each parasite species in the limitation of the host populations is given în Fig. 2A. We obtained the highest degree of parasitism as follows: *Exorista larvarum* 6.60% (with peak parasitism of 66.67% in 1977 for the host larvae in the Băneasa woods and 30.76% in 1978 for the host pupae in the Frasinu woods); *C. concinnata* 2.17% (with peak parasitism of 72.72% in 1977 for the host larvae in the Frasinu woods and 7.53% in 1978 for the host pupae in the same woods) and *B. intermedia* 2.13% (with peak parasitism of 18.57% in 1977 for the host pupae in the Frasinu woods). The other species have a low degree of parasitism (0.006—0.54%).

In our studies, according to the frequency index, only 3 species proved to be permanent parasites: *Exorista larvarum* (38.38%), *C. concinnata* (12.69%) and *B. intermedia* (12.39%); 4 species are accessory parasites: *M. aereus* (7.64%), *H. albicans* (3.13%), *T. atalantae* (2.97%) and *P. uliginosa* (1.22%), while the other species are accidental parasites (Fig. 2B).

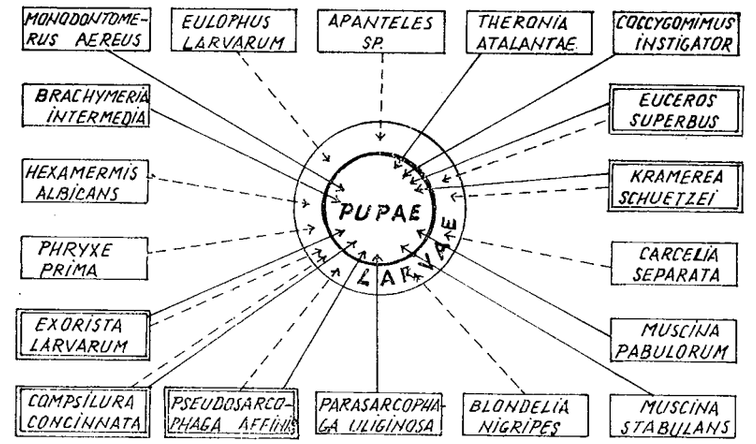

Fig. 1 — The larval and pupal complex of parasites of *Lymantria dispar* L.

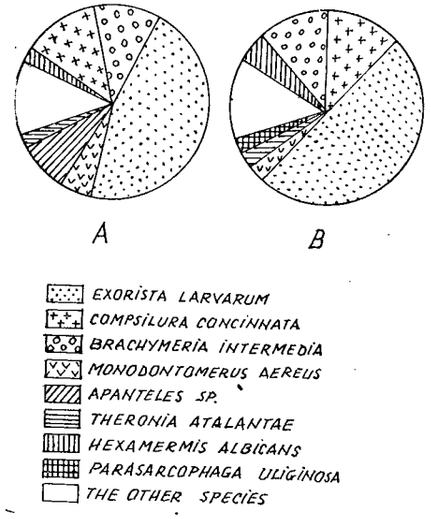

Fig. 2 — *A*. Parasitization index (%) between 1977 and 1980; *B*. Frequency index (%) between 1977 and 1980.

In the seven oak woods from the Giurgiu county, between 1977 and 1980, the parasitic Diptera had the most important share in total parasitism: 33.98—95.11% in the host larvae and 69.23—97 .62% in the host pupae.

The nematode *H. albicans*, a larval parasite of the gypsy moth, had 2.77—59.98% of the total parasitism, while the parasitic Hymenoptera had 0—31.03% in the host larvae and 2.04—30.77% in the host pupae.

Ichneumonids (Hymenoptera) achieved 0.03—2.27% from total parasitism, much less than the parasitic Diptera (Fig. 3). R. W. Campbell [1], [2] reported that ichneumonids favour the parasitization of the gypsy

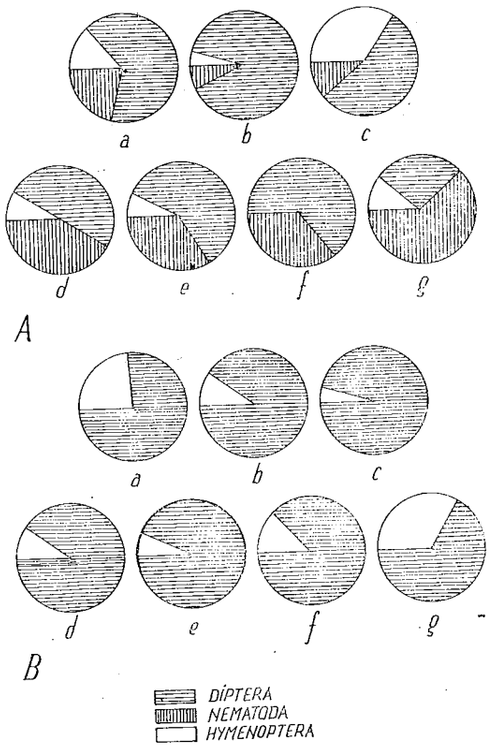

Fig. 3. — *A*. Total parasitization of gypsy moth larvae; *B*. Total parasitization of gypsy moth pupae. *a*. Frasinu woods; *b*. Băneasa woods; *c*. Dăița woods; *d*. Rușii lui Asan woods; *e*. Nebuna woods; . Arbori woods; *g*. B ălășcuța woods.

moth pupae by parasitic Diptera. These ichneumonids sting the host pupae, feeding on the body fluids oozed out from the wound produced by the ovipositor. Through the puncture made by the ichneumonids, the maggots of parasitic Diptera penetrate into the body of the host pupae. This fact has been also confirmed by our study.

CONCLUSIONS

1. During 1977—1980, we obtained from the gypsy moth larvae and pupae, 17 species of the entomophagous parasite insects and one species of parasitic nematodes from seven investigated oak woods in the Giurgiu county.

2. Five species of the natural enemies are both larval and pupal parasites: *E. superbus*, *Exorista larvarum*, *C. concinnata*, *P. affinis* and

K. schuetzei; six species are only larval parasites: *Apanteles* sp., *Eulophus larvarum*, *P. prima*, *C. separata*, *B. nigripes* and *H. albicans* and seven species are only pupal parasites: *T. atalantae*, *C. instigator*, *B. intermedia*, *M. aereus*, *M. stabulans*, *M. pabulorum* and *P. uliginosa*.

3. For the first time in science we obtained from the gypsy moth the following parasites: *Euceros superbus* Kriechb., *Parasarcophaga uliginosa* Kram. and *Phryxe prima* B.B.M.

4. Of the two parasitic Diptera species with a high frequency index, *Exorista larvarum* and *C. concinnata*, the former is more frequent as larval parasite and the latter as pupal parasite.

5. The parasitic Diptera represent the group of the natural enemies with the highest degree of parasitism of the larvae and pupae of *Lymantria dispar*.

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LES MOUSTIQUES DE KISANGANI (ZAÏRE). ABONDANCE, FRÉQUENCE ET BIOMASSE

PAR

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Results obtained between February 1978 and April 1979 in the Kisangani region (Zaïre), concerning periodical frequency and variation of mosquitoes, are presented. This study, based on a wide documentation, concludes that the average biomass of mosquitoes is of 0.51 mg/ind.
Eighteen species have been collected out of which *Aëdes* (*Aed.*) *cumminsii* and *A.* (*St.*) *simpsoni* are new in the region and *Eretmapodites hamoni* is new for the Zaïre fauna in general. *Culex pipiens quinquefasciatus* is the most common in tropical Africa.

Les premières observations concernant les moustiques des environs de Kisangani sont celles de Dr Mouchet [11] et de Dr Schwetz [13]; depuis ces études, aucun travail, à notre connaissance, n'a été publié sur ces insectes de cette zone. Nous appuyant sur cette lacune, nous nous efforçons, avec nos possibilités de continuer cette étude, premièrement sur le plan systématique et écologique; deuxièmement, de chercher les voies et moyens d'une lutte efficace contre ces insectes nuisibles et vecteurs des maladies tropicales (Malaria, filariose et de virus africains) [5].

Sur le plan systématique, deux espèces sont nouvelles pour la zone de Kisangani : *Aëdes comminsii* et *Aëd. simpsoni*. Une nouvelle espèce pour la faune culicienne du Zaïre : *Eretmapodites hamoni*, Grj., dont la description est donnée par l'auteur [6] qui l'a découverte pour la première fois au Congo-Brazzaville.

La situation géographique sommaire de cette zone a déjà été décrite [12]. Dans ce travail, nous présentons la composition taxinomique des espèces récoltées à Kisangani en fonction de leur fréquence; par suite les observations faites sur l'abondance des espèces et la fluctuation saisonnière de la biomasse et de la fréquence de ces insectes au cours de la période d'étude de février 1978 en avril 1979.

Les résultats présentés sont ceux obtenus à partir d'une seule technique de capture c'est-à-dire à l'aide de filets entomologiques.

MATÉRIEL ET MÉTHODE

8874 moustiques ont été capturés directement à l'aide de filets entomologiques dans la végétation basse, les petites formations forestières aux abords des habitations, dans la zone de Kisangani entre février 1978 et avril 1979. Huit stations de récolte, donc chacune limitée à une super-

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ficie de 20 ×20 m², ont été retenues. La récolte a été organisée mensuellement à raison de deux fois par station pendant les heures d'activité intense des moustiques adultes, c'est-à-dire de 4 à 6 h du matin, et de 17 à 19 h du soir.

L'abondance a été exprimée par la densité (rapport de nombre de spécimens à la surface totale de 8 stations de récolte).

Le coefficient de fréquence des espèces est établi d'après les auteurs [2], [15] en appliquant la formule suivante :

$$Cx = \frac{\text{n° individus d'une espèce}}{\text{n° total des individus récoltés}} \times 100$$

La biomasse des moustiques est obtenue par le poids sec. L'animal fraîchement tué (flacon à chloroforme) est placé dans une étuve à 95° C pendant trois jours et, par suite, pesé à l'aide d'une balance électronique. La biomasse d'une espèce est calculée en faisant le produit de l'abondance des individus par le poids sec de l'espèce (à l'état adulte). La somme des biomasses spécifiques constitue la biomasse du peuplement des moustiques à un moment donné. Elle est exprimée en mg de matière sèche.

RÉSULTATS

Composition taxinomique (fréquence des espèces)

Dix-huit espèces de moustiques communs sont à ce jour inventoriées dans la zone de Kisangani (Zaïre). Elles représentent les principales sous-familles de culicidae afrotropicales : *Anophelinae*, *Culicinae* et *Toxorhynchitinae* [3], [5], [14]. Leur richesse spécifique est voisine de celle existant dans la région de Brazzaville sur l'île M'Bamou [6] et dans la région forestière de Buamba en Uganda [8].

Les espèces sont réparties en catégories suivant leur fréquence [15] et les résultats sont exposés dans la figure 1 :

Les espèces expansives sont celles dont le coefficient de fréquence est supérieur à 15 % et qui sont largement répandues dans la zone. Dans cette catégorie, on trouve une seule espèce : *Culex pipiens quinquefasciatus*, Say, (31,5 %). Cette espèce, présente l'année entière, fut trouvée pendant toute la période d'étude.

Les espèces localisées sont celles dont le coefficient de fréquence varie entre 5 et 15 %. Dans cette catégorie sont *Culex moucheti* (11,5 %) ; *Culex schwetzi* (13.5 %) et *Aëdes comminsii* (6,4 %).

Les espèces très localisées sont celles dont le coefficient de fréquence est inférieur à 5 %. On notera les espèces telles que : *Anopheles gambiae*, *Eretmapodites quinquevittatus*, *Eretm. hamoni*, *Eretm. chrysogaster* ; *Aëdes aegypti*, *A. africanus*, *A. congolensis*, *A. vittatus*, *A. circumluteolus*, *A. simpsoni*, *Mansonia africanus*, *M. uniformis*, *Toxorhynchites brevipalpis*.

La dynamique des moustiques de cette zone (tableau 1) montre que les espèces du genre *Culex* tiennent une place très importante quant à

leur abondance et à leur fréquence. *Culex p. quinquefasciatus* a 0,87 ind/m² ; *C. schwetzi* (0,37 ind/m²) ; et *C. moucheti* (0,31 ind/m²). *L'Aëdes congolensis* est l'espèce la moins abondante avec 0,04 ind/m². Les autres espèces du même genre ou des genres différents ont une abondance rélative oscillant entre 0,05 et 0,17 ind/m². La biomasse moyenne obtenue pendant toute la période d'étude est de 0,51 mg/ind. Les individus de *Culex p. quinque-*

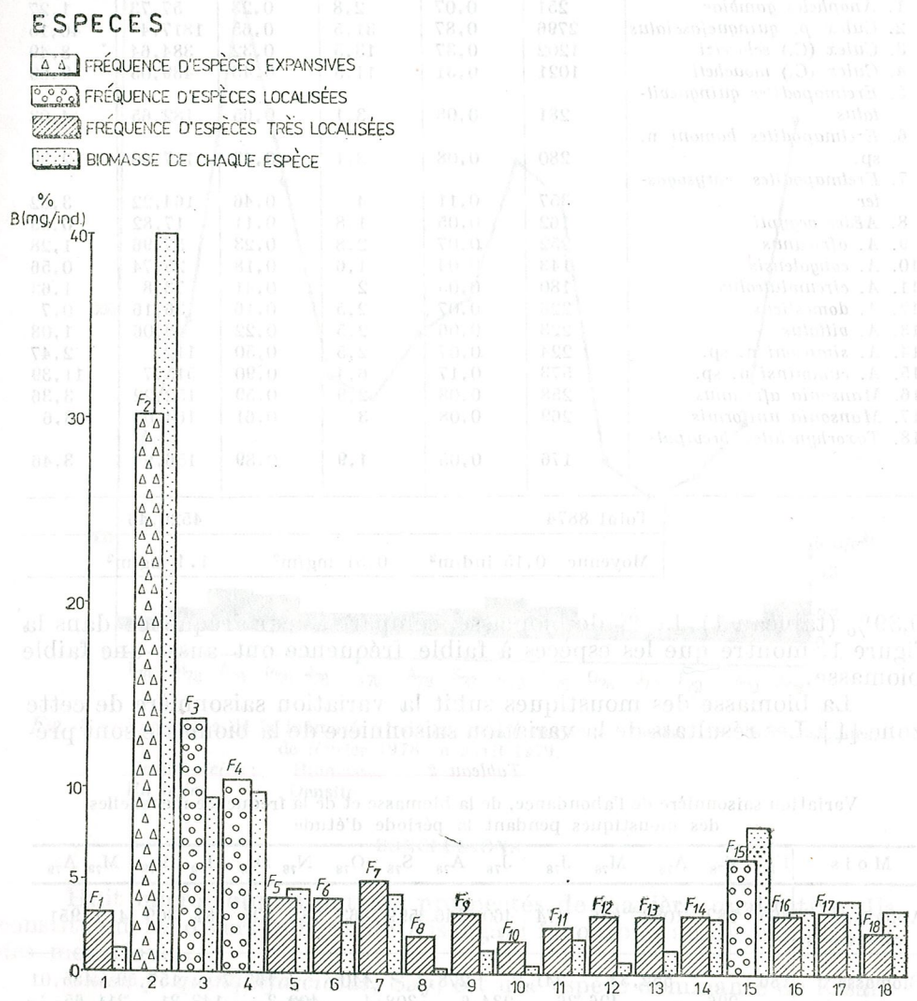

Fig. 1.— Fréquence et bi omasse de ch aque espèce de moustiques récoltés dans la zone Kisangani La numér otation des espèces correspond au tableau 1.

fasciatus présentent une biomasse très élevée de 1817,4 mg ou 40,15 % de la biomasse totale de moustiques récoltés, pendant que les individus d'*Aëdes aegypti* ont une bioma sse relativement faible donc 17,82 mg ou

Tableau 1

Dynamique des moustiques pendant la période d'étude de février 1978 en avril 1979

Espèce	N° d'individus	Abondance ind/m²	Fréquence (%)	Biomasse mg/ind.	Biomasse totale	Biomasse (%)
1. *Anopheles gambiae*	251	0,07	2,8	0,23	57,73	1,27
2. *Culex p. quinquefasciatus*	2796	0,87	31,5	0,65	1817,4	40,15
3. *Culex (C.) schwetzi*	1202	0,37	13,5	0,32	384,64	8,49
4. *Culex (C.) moucheti*	1021	0,31	11,5	0,43	439,03	9,69
5. *Eretmapodites quinquevittatus*	281	0,08	3,1	0,65	182,65	4,03
6. *Eretmapodites hamoni* n. sp.	280	0,08	3,1	0,42	117,60	2,59
7. *Eretmapodites chrysogaster*	357	0,11	4	0,46	164,22	3,62
8. *Aëdes aegypti*	162	0,05	1,8	0,11	17,82	0,39
9. *A. africanus*	252	0,07	2,8	0,23	57,96	1,28
10. *A. congolensis*	143	0,04	1,6	0,18	25,74	0,56
11. *A. circumluteolus*	180	0,05	2	0,41	73,8	1,63
12. *A. domesticus*	226	0,07	2,5	0,16	36,16	0,7
13. *A. vittatus*	223	0,06	2,5	0,22	49,06	1,08
14. *A. simpsoni* n. sp.	224	0,07	2,5	0,50	112	2,47
15. *A. cumminsi* n. sp.	573	0,17	6,4	0,90	515,7	11,39
16. *Mansonia africanus*	258	0,08	2,9	0,59	152,22	3,36
17. *Mansonia uniformis*	269	0,08	3	0,61	164,09	3,6
18. *Toxorhynchites brevipalpis*	176	0,05	1,9	0,89	156,64	3,46
Total	8874				4526,46	
Moyenne	0,15 ind/m²		0,51 mg/m²		1,4 mg/m²	

0,39% (tableau 1). Le % de biomasse, comparé à leur fréquence dans la figure 1, montre que les espèces à faible fréquence ont aussi une faible biomasse.

La biomasse des moustiques subit la variation saisonnière de cette zone [1]. Les résultats de la variation saisonnière de la biomasse sont pré-

Tableau 2

Variation saisonnière de l'abondance, de la biomasse et de la fréquence mensuelles des moustiques pendant la période d'étude

Mois	F_{78}	M_{78}	A_{78}	M_{78}	J_{78}	J_{78}	A_{78}	S_{78}	O_{78}	N_{78}	D_{78}	J_{79}	F_{79}	M_{79}	A_{79}
Abondance absolue	365	582	1083	836	474	460	546	599	863	785	325	281	309	415	951
Biomasse	186	296	552,33	426,36	241,7	234,6	278,4	308,4	440	400,3	165,7	143,31	157,59	211,65	485,01
Fréquence	4,1	6,5	12,5	9,4	5,3	5,2	6,2	6,8	9,7	8,8	3,6	3,6	3,2	4,7	10,7

sentés dans le tableau 2. Les mois d'avril et d'octobre 1978 présentent respectivement une biomasse très élevée, 552,33 mg et 440 mg ; alors que les mois de décembre 1978 et de janvier 1979 ont une faible biomasse,

165,7 mg et 143,4 mg (figure 2). Le poids d'un individu de moustique est négligeable. Pour apprecier le poids significatif des moustiques, la croissance numérique doit être d'environ 2000 individus de moustiques, soit 1,2 kg.

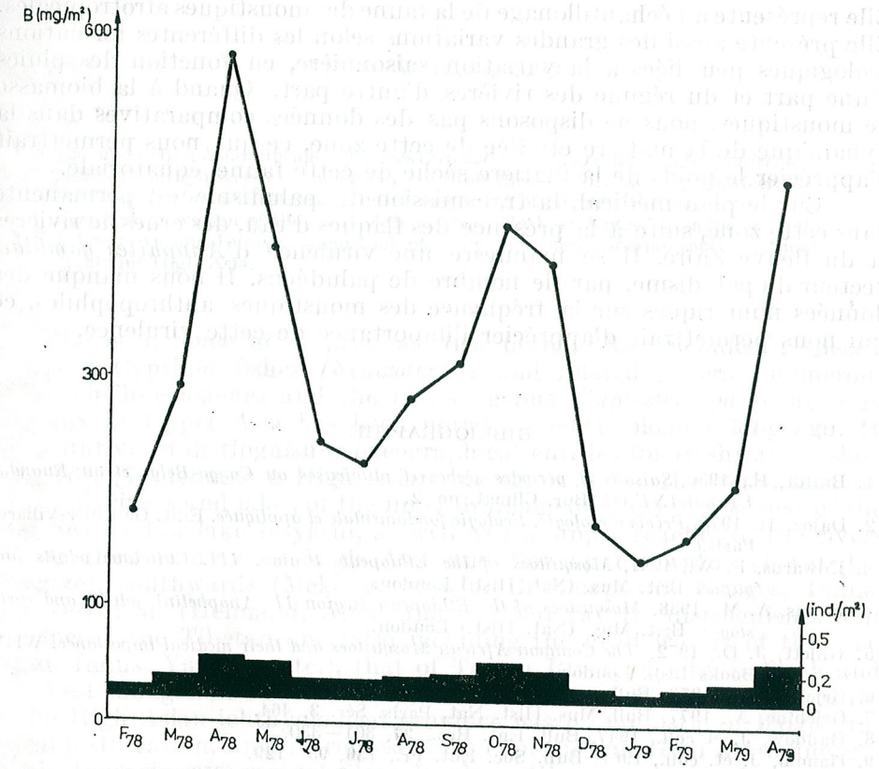

Fig. 2. — Variations de la biomasse et de la densité moyennes mensuelles des moustiques, de février 1978 en avril 1979.
Traits pleins : Biomasse.
En noir : Densité.

CONCLUSIONS

Huit microbiotopes ont été prospectés de manière qualitative. Ils constituent les divers milieux de Kisangani favorables au développement des moustiques.

Culex p. quinquefasciatus, Say, est une espèce dominante de Kisangani par sa population. Elle présente une fréquence de 31,5%. En Mauritanie [10], c'est l'*Anophèles dthali* qui présente une fréquence supérieure. Les résultats exposés concernant les moustiques du Mali [9] montrent que *Anophèles rufipes* est l'espèce la plus répandue (1,45%). Cependant en Afrique centrale, au Congo Populaire, les études menées sur les moustiques de l'île M'Bamou démontrent que les moustiques des genres *Culex*

et *Mansonia* sont fréquents [7]. L'humidité tropicale a une influence sur le préférendum des différents genres et espèces animales (moustiques y compris) quant aux biotopes favorables au développement. Elle favorise le développement massif des moustiques de cette partie d'Afrique.

La faune culicienne de Kisangani constitue notre préoccupation. Elle représente un échantillonage de la faune des moustiques afrotropicales. Elle présente aussi des grandes variations selon les différentes formations écologiques peu liées à la variation saisonnière, en fonction des pluies d'une part et du régime des rivières d'autre part. Quand à la biomasse de moustiques, nous ne disposons pas des données comparatives dans la dynamique de la matière étudiée de cette zone, ce qui nous permettrait d'apprécier le poids de la matière sèche de cette faune équatoriale.

Sur le plan médical, la transmission du paludisme est permanente dans cette zone, suite à la présence des flaques d'eau, des crues de rivières et du fleuve Zaïre. Il se manifeste une virulence d'*Anophèles gambiae*, vecteur du paludisme, par le nombre de paludéens. Il nous manque des données numériques sur la fréquence des moustiques anthropophiles, ce qui nous permettrait d'apprécier l'importance de cette virulence.

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ON THE AFFINITIES AND DERIVATION OF THE AQUATIC FAUNA OF HIGH ASIA

BY

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The main High Asian lineages of fishes (*Schizothoracini, Triplophya, Glyptosternum*) and of aquatic insects (various groups of Stoneflies, Caddis flies, Blephariceridae) have their sisters in East Asia. The aquatic fauna of High Asia is mainly an impoverished derivation of the East Asian ones. Most exchanges of aquatic biota between various Eurasian areas took place by a northern Siberian route, without involving High Asia.

The occurrence of a peculiar fish fauna, that includes a special group of Cyprinid fishes *(Schizothorax* and related genera), numerous Noemacheiline loaches and the catfish genus *Glyptosternum* in the high plateaux of Upper Asia has been noted by ichthyologists long ago. In his tentative to distinguish zoogeographical entities for freshwater fishes, Berg [6], [7] delimits a High Asian subregion of the Holarctis, that includes all rivers and lakes of the inner drainage of Central Asia east of the Aral Sea and of lake Issyk-ul, as well as the upper reaches of the rivers having their sources in High Asia and flowing eastwards (Hwangho, Yangtze), southwards (Mekong, Salwin, Brahmaputra, Ganges, Indus) and westwards (Helmand, Amu- and Syr-Dar'ya). He distinguishes four provinces : the Tibetan one (also including the head waters of the Helmand, Indus, Yangtze, etc.), that of Tarym River, of lake Balkash and the West Mongolian one. Actually, the inclusion of West Mongolia within the High Asian ichthyological province has no faunistic or biogeographical justification, since no representatives of the true High Asian lineages (Schizothoracines, Noemacheiline loaches, Glyptosternon) do range in this area, the fish fauna of which includes two peculiar genera *(Oreoleuciscus* and *Acanthorutilus)* having Holarctic affinities and an endemic grayling, *Thymallus brevirostris*, that belongs to a Holarctic genus, too. This is why I proposed in an earlier paper [2] to consider West Mongolia a distinct subregion of the Holarctic and the true High Asia a subregion of the Sino-Indian region, that also includes East and South Asia.

The fish fauna of the true High Asia consists of the following taxa :

1. The Schizothoracine fishes : nine genera, the largest of which — *Schizothorax (Oreinus)*, *Schizopyge* (= *Schizothorax* by older authors = *Schizothoraichthys)*, *Schizopygopsis*, *Diptychus* (with three subgenera) have a wide range throughout all or most of High Asia, even several of their species ranging in many river basins, the smaller ones have a much more

restricted distribution *(Chuanchia, Platypharodon* and *Herzensteinia* in the east of the north-east of High Asia, *Aspiorhynchus* in the basin of Tarym River while *Schizocypris* is restricted to the south-west of the area, extending from the upper Indus to Seistan, eastern Iran). They constitute a monophyletic taxon, better to be ranked as tribe (Schizothoracini) of Cyprininae. Their sister is the tribe Barbini (subfamily Barbinae by Karaman [12], that includes besides three European and West Asian genera *(Barbus, Capoeta, Aulopyge)* also *Sinocyclocheilichthys* from the middle Yangtze (unpublished data).

2. A peculiar group of Noemacheiline loaches, including 60 or so described species and numerous still undescribed ones; in spite of great differences, above all in the degree of development of the second chamber of the air bladder, which induced Rendahl [13] to distinguish several subgenera, these species share two important common characters — the total absence of scales and a peculiar type of sexual dimorphism, which prove their common origin. They are now grouped in a distinct genus, the right name of which is *Triplophysa* [3]. Its range is slightly smaller than that of Schizothoracini, since it does not include eastern Iran and the upper Ganges basin on the southern slope of the Himalaya. The monotypic *Hedinichthys* from the Tarym drainage represents only a specialization of *Triplophysa*. The sister of the pair *Triplophysa-Hedinichthys* is *Orthrias*, the range of which extends from northern East Asia throughout Siberia to Europe and West Asia.

3. Another genus of Noemacheiline loaches, *Oreias*, that includes three species in the basin of the upper Yangtze and another, more distantly related to the first three, in the basin of Syr- and Amu-Dar'ya [4]; the genus is apparently absent from the intermediate area in Tibet. It seems closer to the mainly South Asian *Schistura*.

4. The catfish genus *Glyptosternum*, that includes three species, ranging in Tarym River, Tibet, upper Brahmaputra, Syr- and Amu-Dar'ya, being absent from the upper Yangtze and Hwangho. According to Hora and Silas [10] it belongs to the seven genera of the "Glyptosternoid group", that centers in Yunnan and adjacent areas at the limits of High and East Asia, also ranging in the north of Burma, Thailand and India.

Hence, the two largest groups of High Asian fishes have sisters ranging in East Asia, Siberia, Europe and West Asia, both being more speciose in the last area. Since the family Cobitidae and the subfamily Cyprininae are by far more differentiated in East- and South Asia, being poorly represented in Europe and absent from North America, it can be assumed that the two lineages were originally East Asian, having later dispersed over Siberia to Europe, this dispersal being younger than the separation of their High Asian sisters, the Schizothoracini and *Triplophysa*. The extinction of Barbini in Siberia is a quite young, Pleistocene event. Also *Glyptosternum* belongs to a prevailing East Asian lineage, only *Oreias* having South Asian affinities.

The biogeographically most significant groups of aquatic Invertebrates — Crayfishes, Prosobranchiate snails, true freshwater mussels (Unionacea) — are absent from High Asia (except a few widely ranging Holarctic of Palaearctic species of *Valvata)*. The pulmonate snails include species (several of them endemic) of the widely ranging *Lymnaea*, *Anisus* and

Planorbis [17]. The fauna of epigaean asellids and gammarids of the area is practically unknown. Hypogaean higher crustaceans were recorded only from the vicinity of lake Issyk-kul, at the limits of High and West Asia. They include species of the primary freshwater and prevailing East Asian *Eobathynella* and of the primary marine *Microcharon* and *Bogidiella* [8], both of which have a mainly Tethyian range. Marine, e.g. Tethyian affinities also have the hypogaean Foraminifera of the area [8]. Biogeographically significant are the aquatic insects of High Asia, three groups of which are here analyzed.

Plecoptera (Stoneflies): some High Asian species belong to widely ranging genera, such as the Holarctic, East and South Asian *Amphinemura*, the Holarctic and East Asian *Capnia*, the Palaearctic and East Asian *Nemoura*, none of which range in India, except its Himalayian areas. Rather many genera are exclusively East and High Asian: *Tylopyge* (most relatives of which range in East and in southeastern Asia), *Rhopalopsole*, *Mesonemoura*, *Mesoperlina* (extending westwards to Iran and Turkestan), *Gibbosia*, *Chinoperla*, *Perlodinella*. Others also range in North America, above all in the Rocky mountains: *Pteronarcys*, *Mesocapnia*, *Paraleuctra*, *Eucapniopsis*, and *Suwallia*. The few genera endemic to High Asia have East Asian or also North American affinities: *Cryptoperla* (related genera also living in southeastern Asia, but not in peninsular India), *Mesyatsia*, *Neofilchneria*, *Cryptoperla*. Much less numerous are the genera showing rather European affinities: *Xanthoperla*, ranging from Europe to Kashmir but apparently not in other areas of High Asia, *Filchneria*, extending from the Caucasus to High Asia and finally the speciose *Leuctra*, the distribution of which is typical Holarctic, also living in West and High Asia but not in East Asia. A single genus has southern Asian affinities: *Indonemoura*, that ranges in the Greater Sunda islands and from Malaya to the eastern Himalayas, but apparently not in East Asia (and neither in India, except its Himalayian areas) [11], [18], [19].

Most Trichoptera from High Asia also have strong East Asian affinities. Genera having an exclusively or almost exclusively East and High Asian distribution are: *Arctopsyche*, *Eodinarthrum*, *Stenopsyche* (with one isolated species in Central India and another in Thailand), *Uenoa* (Japan to Punjab), *Phryganopsyche* (Japan to Assam; this genus represents a family of its own), the whole tribe Micropsychini (Limnephilidae) and several species-groups of *Rhyacophila*. Other genera and higher taxa have a more wider range, also occurring in North America (above all in the west): *Parapsyche*, *Himalopsyche*, *Neophylax Philarctus*, *Eubassilissa*, the subfamilies Dicosmoecinae and Pseudostenophylacinae of Limnephilidae, the pair *Allagapetus* (High Asia and Turkestan) — *Anagapetus* (western North America), several species groups of *Himalopsyche*, the family Limnocentropodidae (genus *Limnocentropus* in Burma, East and High Asia *Yphria* in California) [9], [14—16]. East Asian affinities also have two small genera apparently endemic to High Asia *Oopterygia* and *Neurocyta* (both of Phryganeidae) [9].

Much less numerous are the High Asia caddis flies having European affinities: *Larcasia* (Spain and southern slope of western and eastern Himalayas), *Micropterna* (Europe, West and High Asia, only slightly extending to East Asia) while the genus *Apatania* (Limnephilidae) ranges in Siberia,

the north of High Asia, reaching neither to the southern slope of the Himalayas, nor to East Asia [9], [14], [15].

The genera of Blephariceridae ranging in High Asia are *Philorus* (shared with East Asia and western North America), *Agathon* (same range and also in Siberia), *Blepharicera* (Holarctic and Oriental, the High Asian species being closer to the East Asian and to the western North American ones) and the endemic *Tianshanella* [1].

These data clearly show that most lineages of aquatic insects from High Asia, like those of fishes, have their closest relatives in East Asia. Most data on High Asian water insects refer to those from the southern slope of the Himalayas, e.g. from the High Asian areas closest to India; almost nothing is known about the aquatic insects from the northern half of High Asia, the East Asian affinities of which probably are even better marked.

Although including some speciose genera, the High Asian aquatic fauna is much less diversified that the East Asian, South Asian and even than the European ones, since it consists of much fewer lineages. Numerous genera, subfamilies, etc. of aquatic animals which are well represented in East and /or South Asia are altogether absent in High Asia.

The disjunct of continuous occurrence of a number of lineages in Europe, East Asia and North America, some of which consists of closer related (e.g. recently differentiated) species proves that an active phenomenon of faunal exchange between these areas occurred during the Caenozoic. Arguments were advanced, above all by ichthyologists, that these exchanges took place through a northern, Siberian route, without involving High Asia [5], [6], [2]. Only a few lineages of aquatic insects (e.g. *Larcasia*, *Micropterna*, *Filchneria*, *Xanthoperla*, etc.) could disperse directly between the mountains of southern Europe and High Asia, mountain ranges offering better dispersal possibilities to these animals, even in the absence of river captures, than to fishes, molluscs and higher crustaceans.

The present-day aquatic fauna of High Asia can be considered an empoverished derivation of the East Asian one, some taxa having undergone an active process of speciation and adaptive radiation (e.g. the Schizothoracini and *Tryplophysa*). The area seems not to have been involved in the major faunal movements within Eurasia, in spite of its central position and of the fact that it lies close to the zone where the collision between the Angaran fragment of Laurasia and the former components of Gondwanaland (India, perhaps also southern Asia and even East Asia) took place. Yet the possibility is not excluded that the aquatic fauna of High Asia once was more complex than now, having also included lineages which later became extinct, surviving in East and South Asia, in East Asia and Europe, etc.

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ZYTOPHOTOMETRISCHES STUDIUM DER REDIFFERENZIERUNG WÄHREND DER BILDUNG DES REGENERIERUNGBLASTEMS BEI *DUGESIA GONOCEPHALA* (DUGÈS)

VON

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DNA in regenerating blastomers from normal *Dugesia gonocephala* (2n = 16) was measured cytospectrophotometrically in two wave-lengths and after the first day of sectioning, during 3–4 days, with actinomycin, cycloheximide and mitomycin. All the experiments gave the same images of the heterogeneity of the blastema cells. Thus the dynamics of blastema formation in planarian worms indicates a dedifferentiation of the cells, followed by a later redifferentiation.

In den letzten Jahren wurde die Regenerierung der Planarien Hauptthema zweiter Bücher [1], [2] und einem Syntheseartikel [3], in denen zwei verschiedene Auffassungen dieses Phänomens ausführlich dargelegt sind. Entsprechend der "Theorie der Neoblasten", die von E. Wolff und seiner Schule experimentell begründet wurde, besteht das Blastem ausschließlich aus embryonalen Reservezellen [4], [5], die Neoblasten genannt werden; sie bleiben während des ganzen Lebens undifferenziert und als freie Zellen im Parenchym verbreitet. Die Neoblasten sind die einzigen Zellen der Planarien die infolge einer Verletzung sich durch Teilen und Differenzieren in Zellen verschiedener Gewebe verwandeln können und somit die verletzten Teile wiederherstellen [4], [5], [11], [14]; dabei wird es von der "Wanderung" dieser Zellen gesprochen.

Dieser Theorie entgegengesetzt steht E. Schultzes Auffassung über die "Dedifferenzierung" nach einer Verletzung, ein umgekehrter Vorgang der Differenzierung infolge dessen die Zellen verjüngen; sie gewinnen andere Fähigkeiten und antworten den verschiedenen Induktionsvorgängen durch eine neue Differenzierung. Aufgrund dieser Theorie wurde die dynamische Regenerierung sowohl durch Photonen- und Elektronenmikroskopie, als auch durch Mikrodensimetrie studiert.

MATERIAL UND METHODEN

Die *Dugesia gonocephala*-Exemplare wurden aus dem Gebirgsbach Tufa (Cumpătul-Sinaia) entnommen. Sie wurden post-aurikulär durchgeschnitten und je 10 Exemplare in Petri-Schachteln (Durchmesser 12 cm) mit 35 ml Brunnenwasser verteilt. Das Wasser wurde täglich ersetzt. Es wurden drei zytostatische Substanzen (Mitomyzin, Cycloheximid und Aktinomyzin) hinzugefügt, die die Nukleinsäure beeinflußen und die untersuchten Exemplare wurden mit Zeugenexemplaren verglichen. Nach

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3—4 Tagen wurden die Exemplare in einem kalten Helly-Gemisch fixiert, das die Tiere in einer ausgedehnten Form behalten. Anschließend wurden sie schnell einparafiniert und 6 μ dick sektioniert. Es folgte die Feulgen-Reaktion durch eine Hydrolyse bei der Zimmertemperatur in 5M HCl/35 min, dann eine Spülung in destiliertem Wasser und die Färbung in einer nach Graumann [10] zubereiteten Schiff-Lösung. Die Messungen wurden mit einem Zeiss-Zytospektrophotometer in zwei Wellenlängen ($\lambda = 498$ und $\lambda = 560$ nm) durchgeführt und die Berechnung erfolgte nach E. & R. Rasch [10], wobei $M = nK_2 = AE_2^c$ (Grundfläche der Chromatophoren + Extinktion, durch E_2 korrigiert).

ERGEBNISSE

In allen Fällen wurde innerhalb 3 Tagen die Bildung eines Regenerierungblastems festgestellt. Die wichtigsten Vorgänge dieses Phänomens werden wie folgt zusammengefaßt:

a. Das Epiderma dedifferenziert sich, verliert die Rhabditen, die Kerne werden plattgedrückt, was nach der Wanderung der Epidermzellen und der Bedeckung der Sektionsoberfläche stattfindet. Die Bedeckung der Sektionsoberfläche mit Epiderma ist eine wesentliche Voraussetzung für die Bildung des Regenerierungblastems im Falle einer Amputation.

b. Im Sektionsteil erscheinen Nekrosen, die insbesondere die Neuronen und Gonocyten angreifen.

c. Die Basalmembran löst sich auf und versursacht eine Auseinandersetzung der verschiedenen Gewebe, die somit mobil werden und, indem sie zur Sektionsoberfläche wandern, bilden sie durch ihre Dedifferenzierung das Regenerierungblastem. Um die Basis des Blastems erscheint eine Reihe von Zellpaketen: außenseitig die Muskelzellen, die Pigmentzellen und die verschiedenen Rhabditenzellen, die ihre charakteristische Struktur verlieren, zentripetal wandern und den äußeren Teil des Regenerierungblastems bilden. Unter dieser Schicht wandert eine zweite Zellengruppe, die entweder aus Gonocyten und vitelogenen Drüsenzellen, oder aus Darmzellen und Zellen mit erythrophilen Körnchen besteht. In die ventrale Zone der Amputationsstellen wandern verschiedene Arten von Drüsenzellen.

Nach erfolgter Färbung der parasagitalen Sektion durch die Planarien mit gebildetem Blastem mittels Masson-Goldner-Trichrom, erscheint die Basalmembran gleich einem grünen Netz, in dessen Maschen sich die Zellen der äußeren Schicht befinden: die Muskelzellen, die Pigmentzellen und die Zellen mit Rhabditen, deren Dedifferenzierung beginnt.

d. Im Zytoplasma einiger dedifferenzierten Zellen des Blastems bleiben noch Teile von Myofilamenten, Rhabditen und von erythrophilen Körnchen. Eine allgemeine Charakteristik dieser Zellen ist die vorwiegende Anwesenheit des basophilen Zytoplasmas und der großen Nukleolen.

e. Wie es schon früher gezeigt wurde [9], wandern unter den dedifferenzierten Zellen auch die sogenannten freien Neoblasten verschiedener Größen und Ploidiegrade.

f. Die cytophotometrischen Messungen an den Blastemzellen haben ebenfalls verschiedene Ploidiegrade gezeigt; vorwiegend sind sowohl bei

den Zeugenexemplaren, als auch bei den behandelten Exemplaren DNA-Mengen/Kern festzustellen, die den 2C-Werten im allgemeinen, jedoch auch den 3C-, 4C- und 6C-Werten entsprechen. Es gibt auch eine 1C-Klasse bei den Zellen die das Ende einer Differenzierung erreichten und sich nicht dedifferenzieren können. Sie werden nicht als piknotische Zellen betrachtet, da diese bei unseren Messungen nicht in Kauf genommen wurden.

DISKUSSION

Die Verwendung der zytostatischen Substanzen während der Bildung des Regenerierungblastems bei Planarien beweist einerseits, daß dieser Vorgang sogar in Abwesenheit von Mitosen (order Amitosen) durch das einfache Wandern einiger Zellen aus dem Tierkörper möglich ist und andererseits, daß eine Transkription und eine Translation der betreffenden Zellen in dieser Bildungsperiode des Blastems nicht nötig sind. Übrigens

Tablle 1

Die C-DNA-Menge für die Zeugenexemplare und für die mit Aktinomyzin, Cycloheximid und Mitomyzin behandelten Exemplare

CDNA	6	5	4	3	2	1
Zeugen	2	0	6	21	62	6
Cycloheximid	0	2	6	18	65	9
Mitomyzin	1	0	2	33	56	4
Actinomyzin	3	5	4	13	29	5

Tabelle 2

Die Anzahl der Zellen (%) der Zeugenexemplare und der mit Aktinomyzin, Cycloheximid und Mitomyzin behandelten Exemplare

Zeugen	2,06	0	6,18	21,6	63,9	6,18
Cycloheximid	0	2	6	18	65	9
Mitomyzin	1,04	0	2,08	34,38	58,33	4,17
Aktinomyzin	5,08	8,47	6,78	22	49,15	8,47

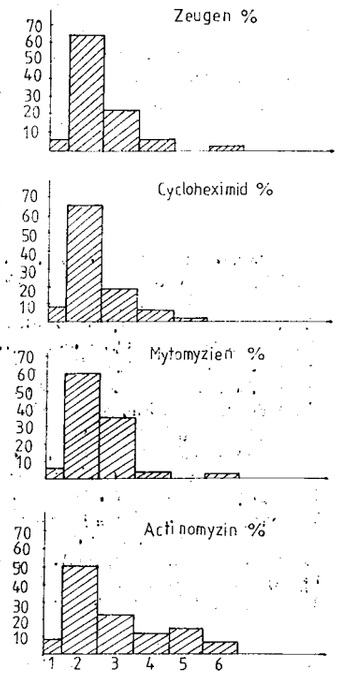

Abb. 1. — Die Variation der DNA-Menge in den Kernen der Regenerierungblastem-Zellen.

ist auch die Integrität des Mitochondrien-DNA keine Voraussetzung für die Bildung des Blastems, da bei den mit Ethidiumbromid behandelten Exemplaren die Amputationsstelle heilen kann und bei den abgeschnittenen

Enden der verschiedenen Organe können eine Dedifferenzierung und sogar die Bildung eines kleinen Blastems stattfinden.

Hingegen ist der allgemeine Metabolismus gestört und die Parenchym-, Darm-, Muskel- und Drüsenzellen füllen sich mit Glykogen und mit anderen Polyzachariden (originale Feststellungen). Interessant ist auch die Tatsache, daß, sowohl bei den Zeugenexemplaren, als auch bei den behandelten Exemplaren die Hystogramme das gleiche Profil vorweisen — was wir während unserer Experimente beobachtet haben. Im allgemeinen wird es angenommen, daß das Aktinomyzin die Zellen in den G_1- und G_2-Phasen und das Cycloheximid in der G_1-Phase blokkieren und daß das Mitomyzin die DNA-Synthese bei den Eukarioten hemmt. In all diesen Fällen können wir annehmen, daß die langfristige (3—4 Tage) Behandlung mit Antibiotika die Zellentypen-Zusammensetzung des Blastems unverändert läßt (Tabellen 1 und 2).

Die Hystogramme zeigen keine Zellen mit proliferativen aktiven Zykeln und das Blastem hat keine Restitutionskerne (Abb. 1), da sie regelmäßig sind und, sowohl bei den Zeugenexemplaren, als auch bei den behandelten Exemplaren dieselben C-DNA-Klassen (1C, 2C, 4C, 6C), jedoch mit geringen nummerischen Variationen vorweisen. Das beruht auf der Tatsache, daß die Kerne der verschiedenen Zelltypen des Tierkörpers verschiedene DNA-Mengen besitzen, was der Ausdruck entsprechender Polyploidiegrade ist, wenn wir die Anzahl der Nukleolen, das Zellvolum und die DNA-Menge in Betracht ziehen.

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ULTRASTRUCTURAL MODIFICATIONS OF THE LIVER IN ROUS SARCOMA (SCHMIDT-RUPPIN) BEARING CHICKENS TREATED WITH "G" INHIBITOR. BIOCHEMICAL INVESTIGATIONS IN LIVER HOMOGENATES AND SERUM

BY

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Electron microscopic observations show that the treatment with "G" inhibitor produces minor reparatory modifications in the hepatocytes of Rous sarcoma bearing chickens. Recovery of treated chickens hepatocytes may be due to a diminution of toxins eliminated by tumours following the inhibition of tumour cells activity by the "G" inhibitor or to the direct effect of the substance on the hepatocytes.

INTRODUCTION

The effects of anticancer treatments are investigated not only by direct observation but also by the complex analysis of various organs which diminish the action of tumor toxins or of the metabolites produced after introducing various anticancerous or toxic substances.

The reactions produced by the tumours and the various chemicals introduced in the organism can be estimated by establishing the values of acid phosphatase [1], [5], [6], [10], [11], [19], [20], [22], [24], [28], [29], alkaline phosphatase [2], [3], [7], [12], [27], transaminases [4], lipids [14] and by the electron microscopic modification of the hepatic cells [8], [15 — 18].

Previous papers established certain ultrastructural modifications of hepatocytes under the action of tumours [8], [16], [17], Rous sarcoma on chicken [16], and the hepatocytes reaction under the influence of some substances [13], [15], [18].

The present paper purposes a convergent analysis of the effects of "G" inhibitor on the liver by studying the hepatocytes ultrastructure and the variations of acid and alkaline phosphatase, of TGP and TGO, of total proteins and lipids from the liver homogenate and serum in Rous sarcoma bearing chickens.

Inhibitor "G": obtained in the "I. D. Cantacuzino" Institute, Bucharest;
Transaminases: TGP = L-alanin 2-ketoglutarat-aminotranspherase; TGO = L-aspart 2-ketoglutarat-aminotranspherase.

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MATERIAL AND METHODS

30-day old chickens were innoculated in the pectoral muscles with 0.5 ml suspension of Rous sarcoma. After 10 days since the innoculation, the animals were given "G" inhibitor for 5 days on end. They were killed after the last injection of the inhibitor.

In order to develop an ultrastructural study, small fragments of liver were fixed for 2 hours in glutaraldehyde 2.5% (Serva, Heidelberg) in 0.1 M phosphate buffer (pH—7.4) and washed in 3 changes of the same buffer at 4 °C. The fragments were later postfixed for 1 hour at low temperature in phosphate buffer 1% OsO_4, dehydrated in acetone series and embedded in Epon (Serva, Heidelberg). Ultrathin sections obtained at Tesla BS 490 A were double stained with uranyl acetate [30] and lead citrate [25] and examined under a JEM—7 (50 kv) electron microscope.

Total proteins [26], total lipids [23], transaminases-glutamic pyruvic—TGP and glutamic oxalacetic—TGO [26] — and acid and alkaline phosphatases [26] were biochemically assayed in serum and liver homogenate. The liver was perfused with physiological serum and the homogenisation was performed in buffer tris HCl—0.2 M + 0.02 M lactose, pH—7.4 and centrifugated at 4 °C.

RESULTS

Important ultrastructural modifications with destructive character were noticed in all our hepatocytes investigations on Rous sarcoma bearing chickens.

They are expressed by chromatin diminishing, mitochondria alteration, important reduction in the number of granular endoplasmic reticulum cisternae. Some cells show clear depletion of glycogen. Sometimes the invasion of figurate elements is noticed in the hepatic cells. Kupffer cells show an important hydrolasic activity.

The treatment with "G" inhibitor produces the partial recovery of the hepatocytes structure.

The hepatocytes nuclei (Fig. 1) preserve their dimensions and normal shape, or present slight modifications induced by the sarcoma presence. At the same time, we can not speak about chromatin recovery chromatin, because it is in small quantities and displayed at the nucleus periphery, on the internal osmiophyle lamina, just as it is present in the untreated animals. The nucleolus or the nucleoli do not change their shape under the influence of "G" inhibitor, but they present a slightly modified aspect, as the sarcoma determined it; that is it presents clear nets, very evident on an electron-dense background. In general, the nucleus is remarkable by electron-clarity in contrast with the rest of the cell.

The mitochondria show a tendency to recover their structure but there occur many modified areas. Most of them have granular-filamentous material resulted from cristae disaggregation (Fig. 2). Many mitochondria exhibit reorganised cristae (Figs 1 and 2), in the fibrillar-gra-

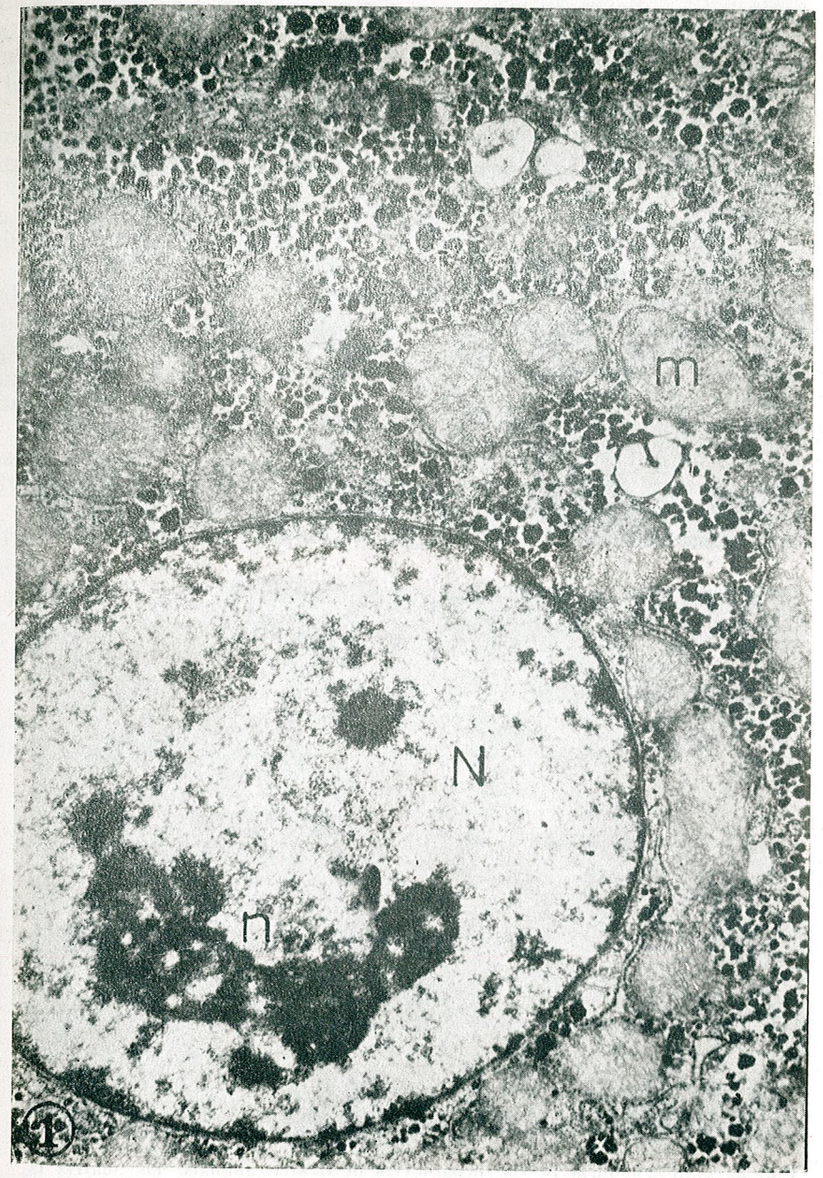
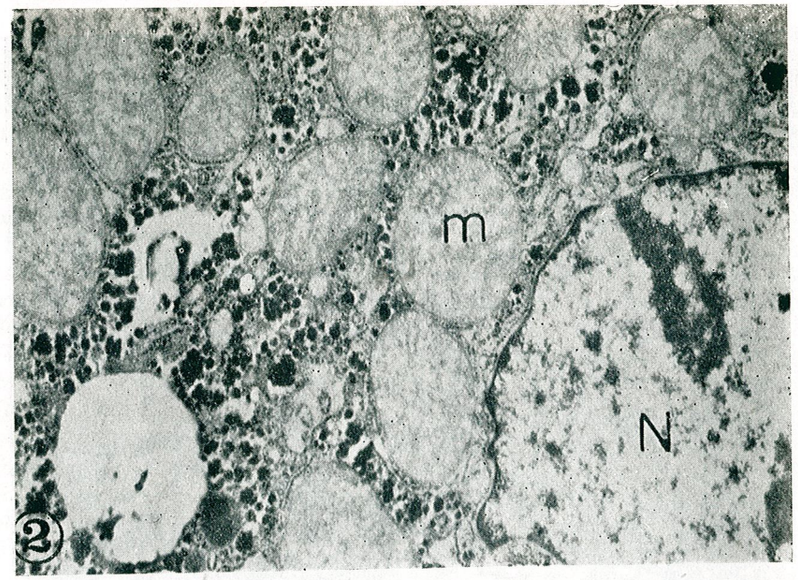


Fig. 1. — Fragment of hepatocyte from a Rous sarcoma bearing chicken treated with "G" inhibitor. The nucleus presents the features of the hepatocytes nuclei in chicken with Rous sarcoma. Mitochondria show a partial recovery of cristal (×14, 700).



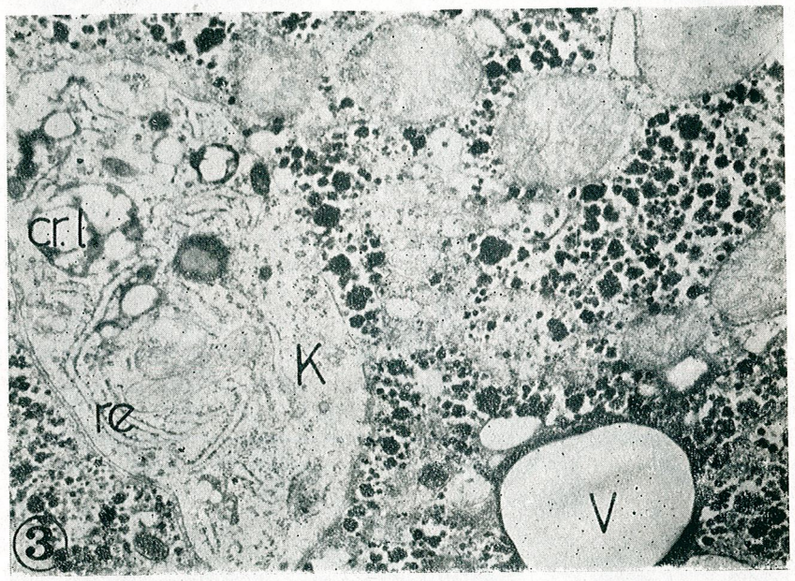


Fig. 2. — Mitochondria in the hepatocytes of treated animals. The matrix is loaded with fibrillary-granular material. Inside can be seen few cristae. Numerous lysis vacuols are also evident (×14, 700).

Fig. 3. — Fragment of Kupffer cell with lysis bodies from treated chickens. Hepatocytes show large electron-clear lysis vacuoles and abundant glycogen (×14, 700).

nular mass. In spite of their restructuration, some of them preserve small slightly modified (Figs 1 and 2) areas of the peripheral membrane. This tendency to restore the mitochondria structure is also present in the strong vacuolated hepatocytes, an aspect preserved after the treatment with "G" inhibitor (Figs 2 and 3).

The electron-clear vesicles with electron-dense thick periphery represent lysis vesicles surrounded by glycogen granules which are under destruction (Figs 2 and 3).

In treated animals, the granular endoplasmic reticulum abundant in untreated hepatic cells is poorly represented and sometimes with incomplete cisternae in the close neighbourhood of the mitochondria.

Glycogen accumulation in cells is the most evident proof of the "G" inhibitor action. The glycogen granules, with their characteristic shape, occupy the whole area among organelles. Sometimes the electron-clear spaces are evident, because they are not completely occupied by the newly formed glycogen; they are clear spaces that under the stress conditions induced by the tumour were determined by glycogen depletion.

Kupffer cells show a well-developed endoplasmic reticulum as well as mitochondria with evident cristae and numerous lysis bodies of a relatively large size (Fig. 3).

The erithrocytes presence inside the hepatocytes was also noticed after the treatment with "G" inhibitor, like those noticed in the liver of untreated animals.

BIOCHEMICAL ANALYSIS

A reduction of transaminases (TGP, TGO) acid and alkaline phosphatases (table 1), is noticed in the liver homogenate and serum of treated animals in comparison with those from untreated animals.

Table nr. 1

Resu lts of biological constants

		Rous sarcoma bearing animals	Rous sarcoma bearing animals with "G" inhibitor
Total proteins	liver homogenate	→	↘
	serum	→	↗
Total lipids	liver homogenate	→	→
	serum	→	↗
Glutamic-pyruvic transaminase (TGP)	liver homogenate	→	↘
	serum	→	→
Glutamic-oxalacetic transaminase (TGO)	liver homogenate	→	↘
	serum	→	↘
Alkaline phosphatase	liver homogenate	→	↘
	serum	→	↘
Acid phosphatase	liver homogenate	→	↘
	serum	→	↘

Total proteins and lipids show t he same reduction in the li v er homo genates from treated animals; in exch ange these constants are increased n the serum of these animals (table 1).

DISCUSSION

Our previous papers described a series of ultrastructural modifications of hepatocytes and Kupffer cells in various vertebrates under the action of some toxins [15], [18] and tumours [8], [16], [17]. Rous sarcoma in chickens induces alterations in hepatocyte structure [16] similar to those produced by hR—18 ascites in rats [17], H—16 melanoma in hamsters [18], carbon tetrachloride in Rana [18], dibenzanthracene in *Chalcides ocellatus* [15], as well as by other toxins [13].

Under the action of the antitumoral "G" inhibitor, a tendency to recover the hepatocytes ultrastructure is noticed; this phenomenon is more evident because of an increased quantity of glycogen in the cells. Moreover, the dosage performed in the liver homogenate obtained from Rous sarcoma bearing chicken treated with "G" inhibitor, show a reduction of transaminases, acid and alkaline phosphatase, lipids and proteins in comparison with the same biological constants of liver homogenate in sick animals. Excepting total proteins and lipids, the same thing is noticed in the serum of treated animals.

The reduction of total proteins value in the liver homogenates from treated animals confirms once more the degree of alteration in cellular structures as well as the decreased capacity of protein synthesis. It is known that in the serum of cancer patients the values of total proteins are low, a reaction which is due to the albumin fraction, but in the serum of treated animals the total proteins have increased values.

Lipids have small values in the liver of treated animals in comparison with the untreated ones. The increased concentration of total lipids in the serum in sick and treated animals would be due to the high concentrations of phospholipids and colesterol.

The biochemical analysis on transaminases (TGP, TGO) is a new confirmation of the fact that "G" inhibitor has a positive influence on the hepatocytes structures and functions. As is known, in the cancerous animals the concentration of some hepatic enzymes is increased. Therefore, the mitochondria alteration is correlated with the increase of both transaminases, especially of TGO. Under the influence of "G" inhibitor, the mitochondria seem to recover their normal structure, explaining therefore the quantitative reduction of transaminases in the liver homogenate and in the serum.

Acid phosphatase, the most active [1] out of the 50 lysosomal hydrolases [19], participates in some destructive processes in the cell related to various pathologies or intoxications [10].

Acid phosphatase, a hydrolasic component of lysosomes [1], [5], [6], [9—12], [21], [22], [24], [28], [29], was not pointed out in the cytoplasm of the treated chicken hepatocytes, but only in lysis bodies of Kupffer cells. Therefore, the processes of self phagocytosis in the hepatic cells are diminished proving that the mucopolysaccharides and glycoproteins destruction is very low [21]. Acid phosphatase as well as the other hydrolases can be consumed, a fact proved by analyses preformed in various pathological conditions [1], [20]. The absence or intense involution of organelles implied in the elaboration of acid phosphatase (RER, Golgi complex) [1], [5], [21], [29], suggest the reduction in the synthesis of this hydrolase.

According to Kasovina [9], [11], the reduced quantity of this enzyme is also due to its reduced transport through the lysosomal membrane.

Alkaline phosphatase, an enzyme of major importance in the transmembrane transport [3], [4], [7], [12], [27], can not be elaborated in the animals hepatocytes, perhaps because of the reduced incidence of Golgi complex [2].

In our case both the stress induced by the tumour in the organism and the "G" inhibitor qualitatively influence hydrolases, transaminases, proteins and lipids.

The relative recovery was demonstrated by electron microscope analysis of hepatocytes of treated chickens; it can be due to an inhibition of the tumoral activity.

At the same time, the reduction in acid and alkaline phosphatases, proteins, transaminases (TGP, TGO) and lipids, leads to the conclusion that there is an incipient recovery of hepatocytes structures, even if most basic biosynthesis structures (microsomes with endoplasmic reticulum, Golgi complex, mitochondria) are considerably reduced and show important signs of structural alteration.

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ELECTRON MICROSCOPIC STUDY OF SOME MALIGNANT RETICULOLYMPHOPROLIFERATIONS

BY

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The results of an electron microscopic study in some cases of neoplastic proliferations of cells belonging to human reticulohistiocytic and lymphocytic systems (Hodgkin's disease, hairy cell leukemia) are presented.
Particular aspects of malignant ultrastructure are presented and discussed as well as electron micrographs which point out the occurrence of some "virus-like" particles in malignant cells and the presence of erythrocytes rosettes constituted around some hairy cells in the spleen. Considerations are also made on the formation of ribosome-lamella complexes.

The early detection of malignant cell proliferations and the correct diagnosis (by identifying the cell type implied in the proliferation process) requires the simultaneous use of classical and modern methods of morphological investigation. Therefore using transmission electron microscopy, information on the ultrastructural modifications characterising a certain type of malignant cell or in the process to become malignant are obtained; useful data on the relations between various types of cells from the malignant lesion, the physiopathological behaviour of malignant cells are obtained. Electron microscopy in transmission, besides other modalities of investigation, is really useful for diagnosing hairy cell leukemia [9], [10] as well as for pointing out some virus-like particles in some cases of human malignant diseases. The examination by means of transmission electron-microscope permits the comparative study of receptor sites on the surface of normal and malignant leucocytes [4]. The scanning electron microscopy technique facilitates the differential diagnosis between lymphocytes type T and B on the one side and the monocytoid blasts on the other side [9], [14]. The techniques presented above, besides some immunological methods, cell cultures, cytogenetical methods as well as cyto-histochemical ones, permit a better knowledge of inmost mechanisms in morphofunctional modifications occurring in malignant cells. Knowing the origin of malignant cells in various neoplasic diseases it was possible to elaborate a classification of malignant cell proliferations in the reticulohistiocyte and lymphocyte systems. These classifications should be appropriate to reality: Rappaport, 1956, Lennert 1973, Lukes and Collins, 1974, classifications (cited by Berceanu, [2]) and Lennert [8].

The purpose of our paper is to present and discuss some modifications occurring in the ultrastructure of blood and spleen malignant cells of patients with Hodgkin's disease and hairy cell leukemia. The recognition of proliferating malignant cell type and an early correct diagnosis permit the application of an efficient treatment.

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MATERIAL AND METHODS

The biological material (bone marrow, blood, spleen) collected from patients with the above-mentioned diseases was worked for histological, cytochemical and electron microscopic study. The specimens for electron microscopic study were prefixed in glutaraldehyde 2% and postfixed in OsO_4 2%. The ultrathin sections were counterstained with uranyl acetate and lead citrate.

RESULTS AND DISCUSSIONS

The electron micrographs obtained by examining spleen of patients with Hodgkin's disease showed the prevalence of some polymorphic cellular types with high N/C relationship. The nuclei of these cells are polymorphic, sometimes deeply incised (Fig. 1); some cells are binucleated. Generally, the nuclei of cells with high N/C relationship have a fine chromatin structure and, they often have 1—2 large nucleoli of a reticular type. Generally, the nucleoli do not have nucleolo-associated chromatin. It is also particular that the cisterna of the nuclear envelope presents evident dilatations on certain portions to which the ribosomes are attached (Fig. 2). Mitochondria of variable sizes as well as ribosomes and endoplasmic reticulum are found in the cytoplasm of these cells. The cytoplasm of some cells is vacuolised. It is performed in two ways:

1. by mitochondria dilatations and cristae disappearance;
2. by exaggerated dilatation of the endoplasmic reticulum.

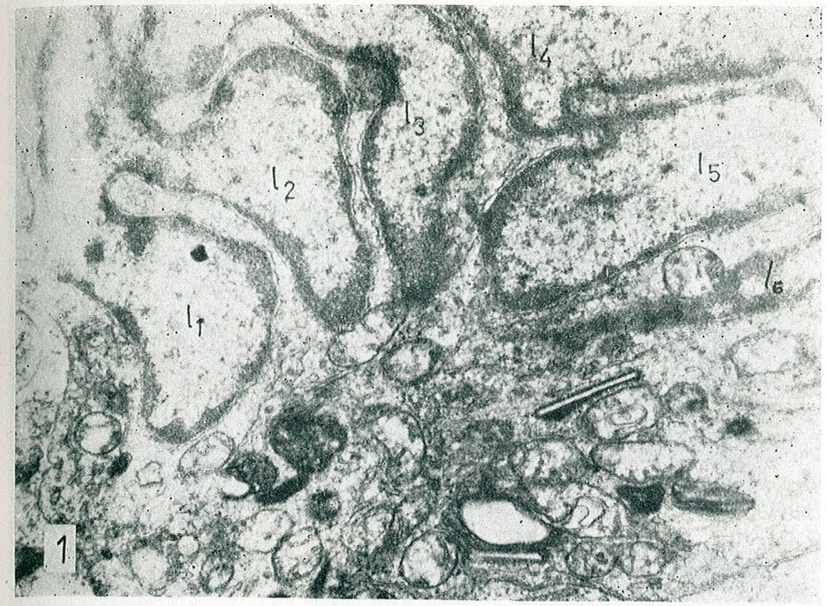
The electron micrographs show that the cytoplasm of some malignant Hodgkin lesions is poor in organelles, justifying therefore the pale aspect of these cells studied with photonic microscope.

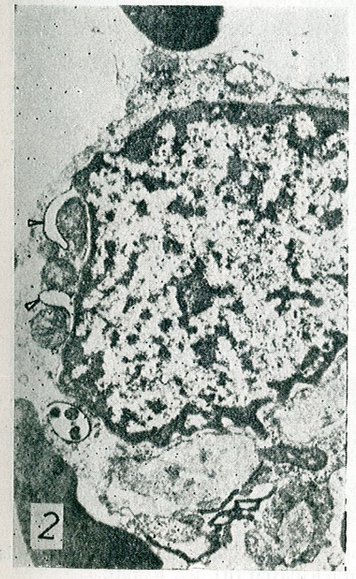
We consider that the large number of cytoplasmic processes and the phagocytary activity in some malignant cells are arguments to support the origin of these cells in the transformed macrophage [1], [7].

Our studies pointed out particles similar to viruses in the spleen malignant cells in two cases of Hodgkin disease. The spleen cells in a patient contain "viroplasma-like" which is generally surrounded by a clear electron microscopic zone. Inside viroplasma, and especially at its periphery, many virus-like particles of 1,200—1,500 Å are noticed (Fig. 3). In the spleen of another patient the cytoplasm presents dense electron microscopic areas, well delimited from the rest of the cytoplasm. Electrondense particles (perhaps virus-like particles whose size ranges between 1,000—1,200 Å) are noticed inside. From a morphological point of view, these electrondense particles are similar to those described by de Harwen [6] in an electron microscopic study of some mesenterical lymphoganglions from mice with lymphoblastic malignant lymphoma.

The ultrastructural aspect of cells containing virus-like particles is the same as of the cells infected with viruses, under a degradation process (destroyed nuclear envelope, altered chromatin structure, mitochondria with partial or totally destroyed cristae).

Inside Hodgkin lesions, the plasmocytes are more numerous than normally, some plasmocytes presenting cytodifferentiation asynchronism.





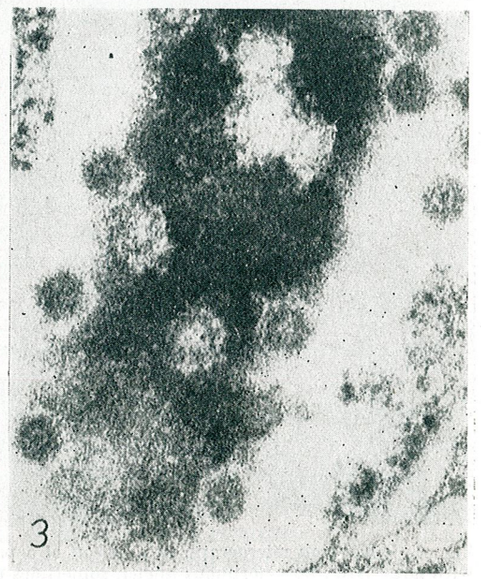
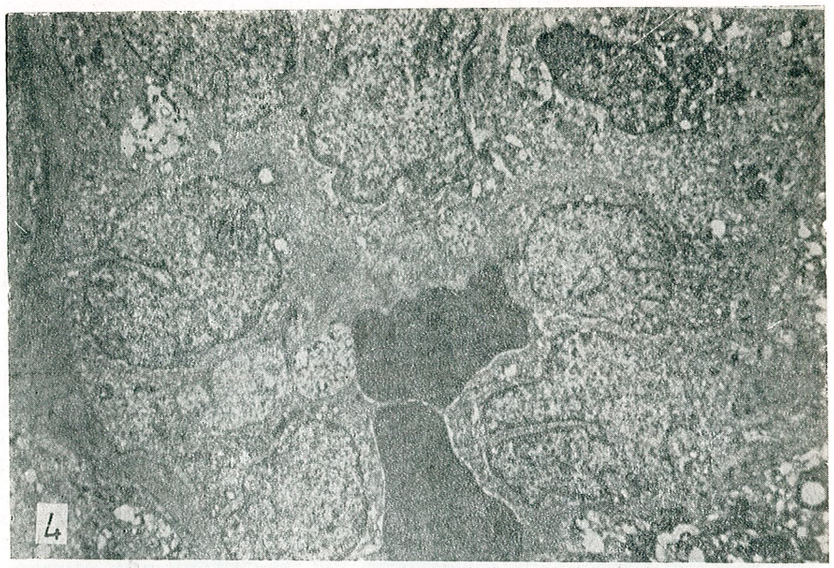
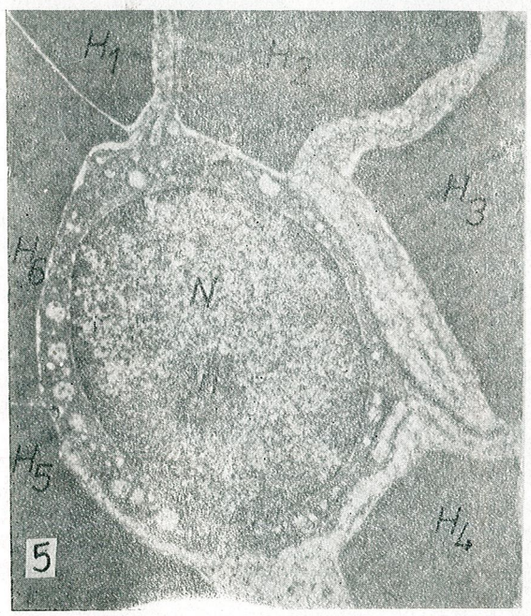


Fig. 1. — Electron microscopic detail in a malignant cell with large N/C; multiple and deeply incised nucleus (l_1-l_6 = nuclear lobes); (spleen, Hodgkin disease; ×50, 400).
Fig. 2. — Cell with dilated nuclear envelope in some portions with the formation of tubular profiles (◁). Ribosomes attach against them (spleen, Hodgkin disease; ×6, 800).
Fig. 3. — Viroplasma like with virus-like particles in a spleen cell of a patient with Hodgkinian spleen lesions (50,000).





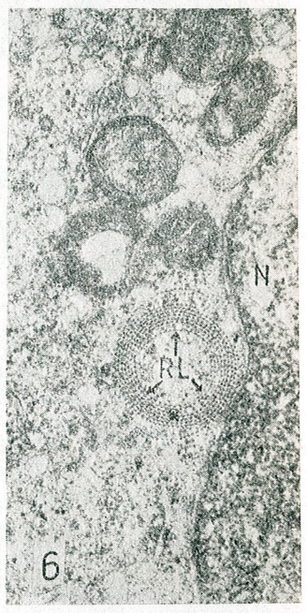


Fig. 4. — Electron micrograph in a spleen of a patient with hairy cell leukemia. Cells with large N/C ; polymorphous nuclei, sometimes nucleolated (n). (× 8,300).
Fig. 5. — Erythrocytes rosette (H_1—H_6) surrounding a hairy cell (large N/C and nucleolated (n) nucleus (N).(× 12,400).
Fig. 6. — Ribosome-lamella complex (RL) in a hairy cell nucleus (N) vicinity. The continuity relation between the external membrane of nuclear envelope and the lamellar component of the ribosome-lamella complex is seen (spleen, hairy cell leukemia ; × 46,000).

The statistical data in literature show that hairy cell leukemia affects men to a greater extent than women. Golomb [5] mentions that only 29% of women are affected by this disease, while Burke [3] mentions 10%. Our statistics shows that seven out of ten patients are men [11].

After a morphological examination of blood and bone marrow in a patient suspect of malignant disease, the existence of some irregularly shaped cells was mentioned. After electron microscopic investigation of blood, a lot of hairy cells were noticed. The cells present many cytoplasmic processes. Sometimes, the processes of the same cells get into contact with each other, and after recombinations with plasmalema, form endocytosis vesicles.

The patient was splenectomised. The electron microscopic examination of spleen shows the existence of numerous hairy cells. As well as those in vein blood, the hairy cells present many cytoplasmic processes. The cells with high N/C relationship represent the prevailing cellular population (Fig. 4). The nuclei of these cells show a high degree of polymorphism (some of them are oval or circular in section, others are deeply incised or convoluted) with a fine chromatin structure. Most of their nuclei have 1—2 nucleoli of a reticular type. Some hairy cells have little cytoplasm. However, most hairy cells have a cytoplasm rich in organelles : dictiosomes, mitochondria, smooth endoplasmic reticulum, many ribosomes. Sometimes, the mitochondria in the cytoplasm of these cells are under a degradation process.

We consider that the detection of pseudosinuses in the spleen of patients with hairy cell leukemia shows an advanced stage in the malignant proliferation [12].

Some hairy cells give a slightly positive reaction to Periodic Acid Schiff (small PAS positive granules uniformly widespread are seen in the cytoplasm). The reaction for pointing out the peroxydasis with Diaminbenzidine and oxygenated water is negative in the case of hairy cells.

As a particular ultrastructural aspect, we mention the fact that, sometimes, a rosette of erythrocytes is established around a hairy cell situated in its normal environment—in the spleen (Fig. 5). Since the erythrocytes rosettes stable in vitro at 37 °C surround the malignant human T lymphocytes [13], we suppose that this ultrastructural aspect visible in the spleen of patients with hairy cell leukemia suggests the origin of hairy cells in the malignant T lymphocyte. However, we do not know the real physiopathological significance of this aspect.

Generally, in the neighbourhood of some hairy cells, the presence of so-called ribosome-lamella complexes was noticed (Fig. 6). Katayama, (1972), cited by Lennert [8], describes for the first time these structures.

Most authors consider these structures as specific for hairy cell leukemia. The origin of ribosome-lamella complexes is not well known [8]. The results of our electron microscopic investigations support the idea that the cytomembranes of these complexes are derived from the external membrane of the nuclear envelope (Fig. 6). In this respect we underline the high frequency of the juxtanuclear position occupied by these struc-

tures. Moreover, in some electron microscopic images we noticed the direct connection between the external membrane of the nuclear envelope and the cytomembranes of ribosome-lamella complex in the neighbourhood of the nucleus. It seems that the redundant membranes derived from the external membrane of the nuclear envelope are displayed in a spiral, with the ribosomes attached at equal distances. The measurement performed showed that the length of a ribosome-lamella complex is of 4,400 nm; the exterior diameter is of 1,000 nm and the interior one of 500 nm. The distance between two neighbour lamellar spirale is of 400 Å, and the electrondense particles are of 200—300 Å, dimensions similar to those of the ribosomes.

In some cell cytoplasm we noticed the existence of some viral matrices with virus-like particles of 1,200 Å.

CONCLUSIONS

1. In tissues with malignant lesions, the cells with high N/C relationship are prevailing. The nuclei of malignant cells have a fine chromatin and are nucleolated.

2. Sometimes, in the cytoplasm of malignant cells, alterations of cellular organelles are produced (dilated mitochondria with partially or totally destroyed cristae; large dilatations of the endoplasmic reticulum and of the nuclear envelope etc.)

3. The hairy cells present particular cytoplasmic processes and ribosome-lamella. We consider probable the derivation of ribosome-lamella from the external membrane of the nuclear envelope.

4. We mention the presence of the erythrocytes rosette established around the hairy cell identified in the spleen of a patient with hairy cell leukemia.

5. Electron microscopic examination pointed out virus-like particles in some malignant cells; therefore, a viral etiology in human neoplastic disease is supported.

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THE MODE OF ACTION OF ANTIBIOTIC PREPARATION A 6.7 ON THE MESSENGER—RNA TRANSCRIPTION

BY

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The "in vitro" interaction between antibiotic preparation A 6.7 and two DNA preparations (pure DNA and DNA extracted from HeLa cell nuclei) was investigated. The comparative analysis of absorption spectra in U.V. light revealed a change in the kinetics of absorption spectra of A 6.7 preparation in the presence of DNA, which suggest a possible specific coupling between the two components. It was postulated that the interaction between A 6.7 and DNA, as observed in this study, plays an important role in the antibiotic mechanism of action.

In a series of previous investigations carried out on HeLa cell cultures we have shown that the antibiotic preparation A 6.7 induced an inhibitory effect on culture development [16] and the synthesis of messenger-RNA [17]. The characteristic pattern of the total cell proteins recorded during the evolution of cell cultures submitted to the drug and reflecting an alteration of protein synthesis appears to be the result of the low messenger-RNA (mRNA) level.

The inhibition of mRNA synthesis and consequently of the protein biosynthesis induced by the cytostatic agent A 6.7, seems to be the result of its interaction with the molecular mechanisms governing the regulation of transcription of mRNA adenylation and maturation, processes which are sensitive to some antibiotic action [8], [10].

In this paper, the results of a preliminary study on the mechanism of action of A 6.7 preparation are presented.

The investigation of the possible action of the drug on mRNA-DNA dependent transcription was achieved by studying "in vitro" the direct interaction between A 6.7 and DNA, determining the difference of the absorption spectra of the components.

MATERIAL AND METHODS

In this study two DNA preparations have been used : one extracted by us from the nuclear pellet of the control HeLa cells, separated from the postnuclear supernatant used for the extraction of cytoplasmic RNA, and another, pure DNA (BDH).

EXTRACTION AND PURIFICATION OF NUCLEAR DNA

Nuclear DNA was obtained according to the methods described by Doerfler [3] and Robinson [15].

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The nuclei resuspended in 0.02M Tris-Hcl buffer, pH 7, 50 mM EDTA have been lysed by adding SDS at a final concentration of 1% to an equal volume of extraction mixture PITC-9 (phenol-isoamylic alcohol — Tris-Hcl buffer—chloroform 100 : 16 : 8 : 100) and agitating for 30 minutes at room temperature. The homogenate was centrifuged for 20 minutes at 10,000 rpm and 2 °C. To the final aqueous phase obtained after four successive reextractions 4M Nacl was added to a final concentration of 0.2M, and an equal volume of absolute ethanol. The DNA was collected on a glass rod. To the DNA redissolved in 0.02M Tris-Hcl, pH 9.1 nM EDTA, 0.5% SDS was added by agitation at room temperature, and then trypsin solution to a final concentration of 300 μg/ml, incubating for 4 h at 37 °C. The extraction with PITC-9 mixture was repeated and the purified DNA was precipitated with ethanol and Nacl for 48 hours at —20 °C.

The DNA separated by centrifugation for 15 minutes at 6,000 rpm and 2 °C, was resuspended in 0.02M Tris-Hcl, pH 7.4, 1 mM EDTA to a final concentration of 1 mg/ml.

The chemical nature of the extracted material was established by the biochemical test with diphenylamine [2].

INCUBATION OF DNA WITH THE ANTIBIOTIC PREPARATION A. 6.7

Aliquots of DNA (1 mg/ml) either pure or extracted from the HeLa cell nuclei were incubated with the antibiotic isolate A 6.7 (0.5 mg/ml) for 15 minutes at 37 °C. Some incubates have been used for the recording of UV absorption spectra and the others were treated with Nacl and ethanol for 48 hours at —20 °C for the precipitation of DNA or of the eventual complex.

The precipitate was separated by centrifugation at 6,000 rpm for 15 minutes, resuspended in buffer and the absorption spectra in UV light have been recorded.

SPECTROPHOTOMETRIC EVALUATION OF DIFFERENCES IN THE ABSORPTION SPECTRA

The interaction between DNA and the antibiotic isolate A 6.7 was followed by comparative analysis of absorption spectra characteristic of drug A 6.7 and of DNA and of the incubates of DNA and A 6.7, recorded with a Spekord spectrophotometer in UV.

RESULTS

Fig. 1 shows the characteristic absorption spectrum of the antibiotic preparation A 6.7 (0.5 mg/ml). Two peaks of maximum absorption at 235 and 280 nm are observed.

DNA (1 mg/ml) spectrum presented in Fig. 2 is characterized by an absorption maximum at 235 nm, a minimum at 260 nm, followed by another maximum at 300 nm; these details appear for both forms of DNA used in the experiment.

Fig. 3 illustrates the absorption spectra recorded with the incubates of isolated DNA from A 6.7, immediately after the lapse of time allowed for the contact between the two components.

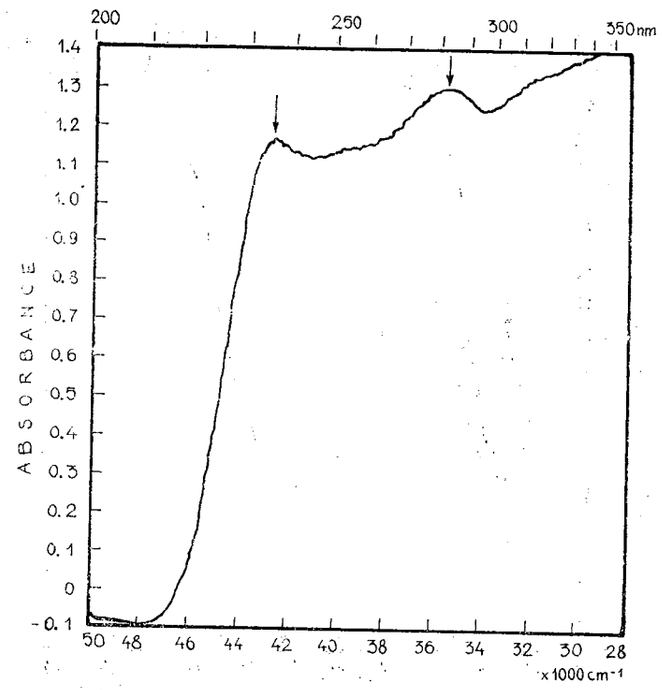

Fig. 1. — Absorption spectrum in UV light of antibiotic preparation A 6.7 (0.5 mg/ml).

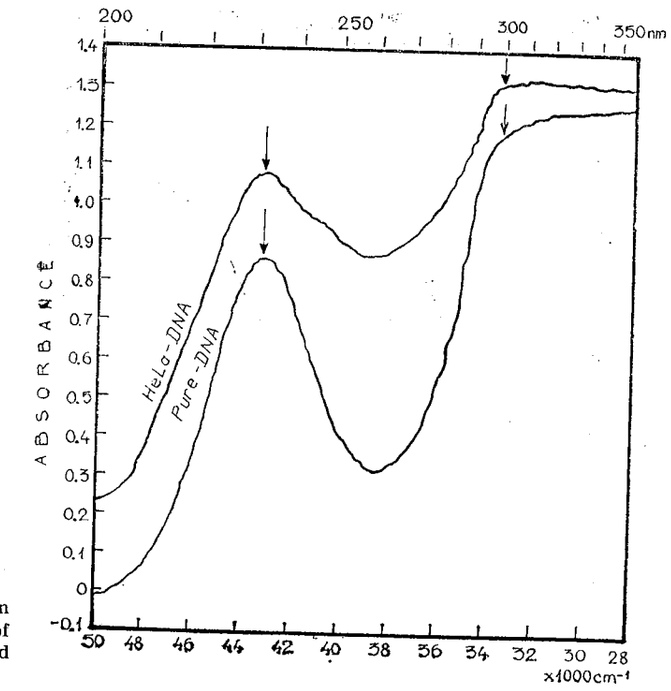

Fig. 2. — Absorption spectra in UV light of HeLa nuclear DNA and pure A (1mg/ml).

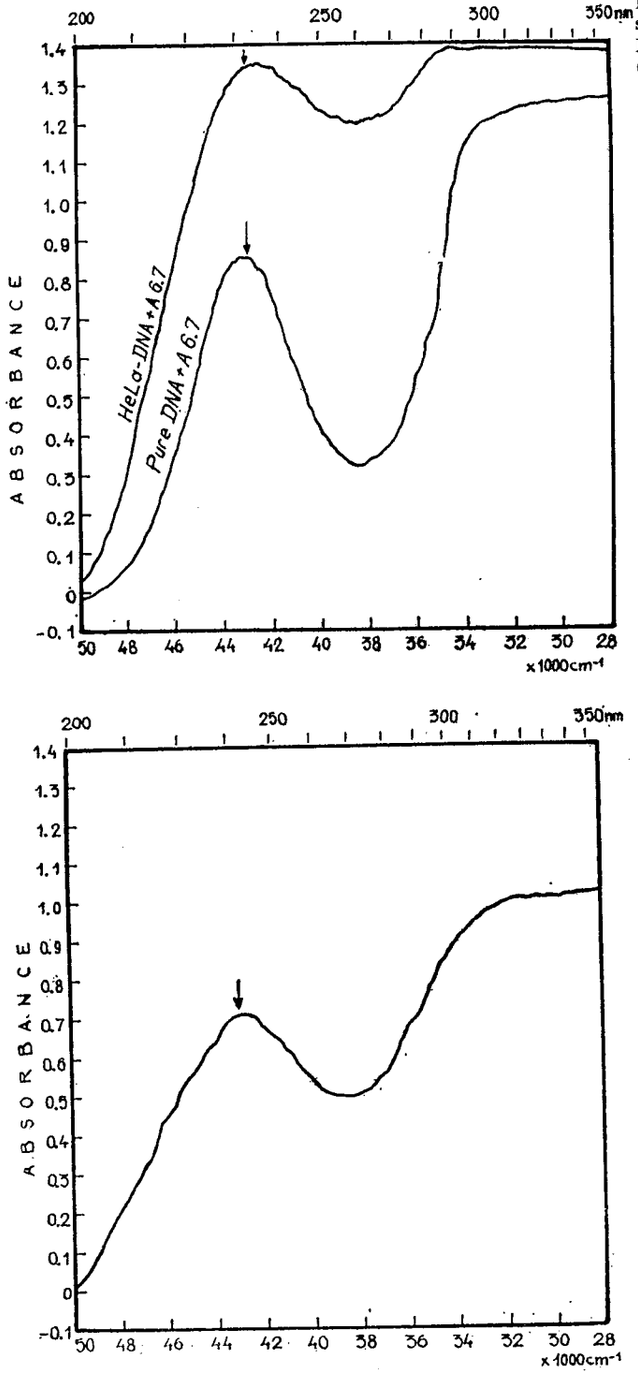

Fig. 3. — Absorption spectra in UV light of DNA (1 mg/ml) — A 6.7 (0.5 mg/ml) mixtures.

Fig . 4. — Absorption spectrum in UV light of DNA-A 6.7 complex. after ethanol precipitation for 48 hours at —20°C,

Regardless of the type of DNA used for the evaluation of a possible direct interaction with the antibiotic preparation the absorption spectra recorded are characterized by two absorption maxima at 235 and 300 nm.

The disappearance of the absorption maximum at 280 nm characteristic of the antibiotic preparation A 6.7 was observed.

As mentioned above, some incubates of DNA-drug have been treated in order to precipitate the eventual complex formed; its absorption spectra is shown in Fig. 4. Again there are two absorption maxima characteristic of DNA at 235 and 300 nm and the absence of a peak at 280 nm specific for the antibiotic preparation A 6.7.

The disappearance of the absorption maximum at 280 nm specific for the antibiotic preparation observed on the spectra of DNA-A 6.7 incubates would plead for a probable coupling of the antibiotic preparation with the DNA macromolecule.

DISCUSSION

Estimation of an altered cellular protein synthesis, correlated with the inhibition of mRNA biosynthesis registered on HeLa cell cultures treated with the antibiotic preparation A 6.7 [16], [17], pointed to the study of the possible mechanism of action of this cytostatic preparation.

Since the quantitative estimation of cellular DNA determined on HeLa cells treated with the drug was not significantly different from the quantity of DNA estimated in control HeLa cells (unpublished data), we appreciated that the mechanism of action of the drug A 6.7 could be represented by a molecular effect on mRNA transcription or an mRNA adenylation and/or maturation rather than the interference with DNA replication.

First, we tried to see whether the mechanism of action of the antibiotic preparation was not represented by an action on DNA-dependent RNA transcription. Therefore, we investigated "in vitro" if a direct interaction between the isolate A 6.7 and DNA was possible.

As shown above, the comparative analysis of absorption spectra in UV of the two separate or incubated components showed a spectral difference which proved to be constant in various experimental conditions and this consisted in the disappearance of the absorption maximum at 280 nm characteristic of A 6.7 isolate incubated with DNA.

This difference in the kinetics of absorption spectra suggested the possibility of a probable coupling of the cytostatic agent with DNA macromolecule, that is of a direct interaction between these components.

The coupling of the drug to DNA could induce blockage of the DNA-dependent mRNA transcription which would thus explain the low level of mRNA biosynthesis and consequently of the cellular protein synthesis.

In this stage, it is difficult to explain the intimate mechanism by which the antibiotic preparation A 6.7 functionally interfere with the complex stages of transcription process. The coupling of the cytostatic

agent to nuclear DNA could block the template function of DNA matrix for mRNA transcription either by preventing the RNA-polymerase coupling or by preventing the movement of RNA-polymerase along the DNA matrix.

This supposition regarding the probable mechanism of action of the antibiotic preparation A 6.7 as a functional interference with the molecular mechanisms regulating the complex stages of transcription is supported by several data in the literature which proved the existence of some antibiotics in antracycline and quinoxaline group which by coupling to DNA block its template function, that is the transcription, thus inhibiting mRNA synthesis.

Comparative studies carried out on different test biological systems (bacteria, KB and HeLa cells) showed that antibiotics such as nogalamycin [1], [13], [14], actinomycin [4], [12], daunomycin and chromomycin [21], rybomycin [11], antramycin [7], quinomycin [18], [19], violamycin [20], adriamycin [6], carminomycin [5], variamycin [9], interact with polynucleotides based on their specificity of base (guanine, adenine, tymine) binding, forming a complex with the DNA matrix by which they block its template function and thus inhibit the enzymatic DNA-mediated mRNA synthesis.

Therefore, it could be considered that the antibiotic preparation A 6.7 is a true and potent cytostatic agent whose possible molecular mechanism of action also consists of blockage of template function of the DNA matrix by specific coupling, thus inhibiting mRNA transcription and consequently cellular protein synthesis.

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ÉTUDE ANALYTIQUE DE L'INFLUENCE D'UN RAYONNEMENT MICROONDE SUR LA MORTALITÉ AVANT ET APRÈS ÉPREUVE, DE SOURIS VACCINÉES ET ÉPROUVÉES PAR DES MUTANTS DE *S. TYPHIMURIUM*

PAR

P. BINDER, PH. ASPEEL, P. DESCHAUX et R. FONTANGES

Mice were immunized against *S. typhimurium* mutants and exposed to 2450 MHz CW microwave radiation in a specially constructed absorbant chamber. Animals were exposed to 20 mW/cm² of irradiant power intensity 4 hours per day during 4 days. Results were exploited according to an original statistical method. Immunizing value of no pathogenic living microorganisms was modified into exposed animals. A mortality level was observed when animals vaccination was performed after irradiation. A modification of survival level into immunized and irradiated animals was also noticed.

1. INTRODUCTION

Les hyperfréquences de plus en plus utilisées dans l'industrie ou, chez le particulier sont d'un emploi qui n'est pas sans poser quelques problèmes quant à leurs effets biologiques [2].

Certains d'entre nous [8] avaient déjà montré qu'il existait une stimulation de la réponse immunitaire de la souris Swiss mâle après une irradiation de 2450 MHz pour une puissance de 10 mW/cm² de surface corporelle à raison de 4h/j. pendant 4 jours consécutifs. Cependant, la dose effectivement reçue par les animaux ne pouvait être que théorique, en raison de l'emploi d'une chambre multimode. D'autres travaux, réalisés cette fois en chambre anéchoique, montrèrent que la protection obtenue après vaccination à l'aide de ribosomes de *Klebsiella pneumoniae* n'était pas modifiée pour des puissances de 5 à 10 mW/cm² [10] alors que l'on pouvait noter [3] une immunostimulation, si cette puissance était de l'ordre de 20 mW/cm². Ces derniers résultats nous ont conduits à étudier de manière approfondie avec analyse méthodique des résultats, l'influence de l'irradiation microonde sur le pouvoir vaccinant, de différents mutants S. de *Salmonella typhimurium*.

2. MATÉRIELS ET MÉTHODES

2.1. LES ANIMAUX

Nous avons utilisé 430 souris Balb/c mâles, âgées de 6 à 8 semaines, pesants environ 25 g, réparties en lots d'environ 20 animaux.

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2.2. CONDITIONS D'IRRADIATION

Les animaux sont placés dans une chambre anechoïque de 1 m³. La puissance appliquée est contrôleé par une sonde NARDA modèle 8201. La fréquence est de 2450 MHz et la puissance réglée à 20 mW/cm². Les animaux sont placés dans des cages individuelles en plastique et exposés à l'irradiation 4h/j. pendant 4 jours consécutifs. La température de l'enceinte est enregistrée en continu ; elle ne dépasse jamais la température ambiante de plus de 4 °C

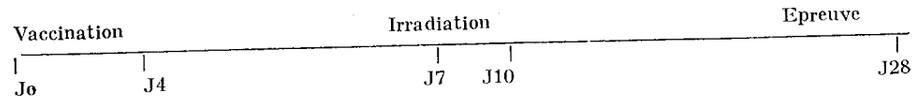
2.3. ETUDE DU POUVOIR VACCINANT

Nous avons choisi comme vaccins les mutants S de *S. typhimurium* Ra, Rc et Re. Les suspensions vaccinantes sont inoculées per os à l'aide d'une sonde oesophagienne à raison de 1×10^4 germes dans 0,2 ml, chaque jour pendant 5 jours de suite, dans du sérum physiologique.

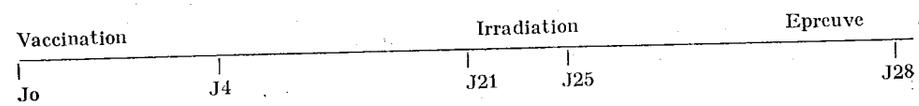
Le germe d'épreuve est représenté par *S. typhimurium* souche CS. Il est administré également par voie orale à raison d'une dose unique d'environ 5×10^4 germes, 28 jours après le début de la vaccination.

2.4. MODALITÉS DE L'IRRADIATION ET RÉPARTITION DES LOTS

Modalité I : L'irradiation débute 48 jours après le dernier jour de la vaccination soit 21 jours avant l'épreuve et se poursuit pendant 4 jours.



Modalité II : L'irradiation débute 16 jours après la fin de la vaccination soit 7 jours avant l'épreuve et se poursuit pendant 4 jours.



Pour chaque mutant, on constitue 6 lots d'animaux.

Les lots 1 et 2 sont des témoins soumis aux mêmes conditions d'environnement que les animaux irradiés. Ils sont placés dans des cages individuelles et dans une enceinte obscure, mais ne sont pas soumis à l'irradiation microonde. Le lot 1 est constitué d'animaux non vaccinés contrairement au lot 2. Les lots 3 et 4 sont soumis à une irradiation selon la modalité I. Seuls les animaux du lot 3 sont vaccinés.

Les lots 5 et 6 sont soumis à une irradiation selon la modalité II. Seuls les animaux du lots 5 sont vaccinés.

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2.5. MÉTHODE D'ÉTUDE ET D'INTERPRÉTATION DES RÉSULTATS

Pour chaque lot, on compte chaque jour, pendant 20 jours, le nombre de survivants après l'épreuve. L'observation des courbes de survie relative permet de distinguer 3 périodes caractérisables par 3 variables.

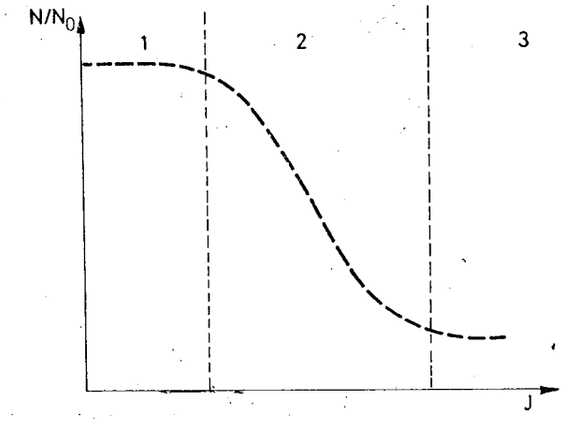

Fig. 1 — Courbe de survie relative théorique.

2.5.1. La période 1 représente la durée d'incubation de la maladie expérimentale. C'est le nombre de jours qui s'écoulent entre l'épreuve et l'apparition des premiers morts. On construit un tableau et on effectue une analyse de variance sur ces périodes d'incubation pour chaque type de mutant et chaque lot.

2.5.2. La période 2 représente la phase de décroissance des échantillons par mortalité journalière liée à la maladie expérimentale. C'est donc cette mortalité journalière qui sera étudiée. Il est possible à priori, de poser comme hypothèses :

1. que le nombre de morts par jour est constant. L'équation de mortalité représentative est alors :

$$Nj = No - K_1 Noj$$

Nj = nombre de morts au jour j,
No = nombre d'animaux éprouvés,
K_1 = constance de mortalité journalière.

2. que la mortalité journalière est constante, l'équation représentative est alors

$$Nj = No\ e^{-K_2 j}$$

K_2 est le taux de mortalité journalière.

3. que le nombre de morts quotidiens est une fonction du temps, sans être liés au nombre d'animaux restants. L'équation représentative devient

$$Nj = No - 1/2\ K_3\ \ No\ \ J^2$$

où K_3 est la constante d'accélération de la mortalité.

4. que la mortalité journalière est fonction du temps et du nombre d'animaux. L'équation représentative devient

$$Nj = No\ e^{-1/2}\ K_4\ Noj^2$$

où K_4 est la constante d'accélération de la mortalité.

On peut remarquer que les équations 1 et 3 ne sont en réalité que des cas particuliers des équations 2 et 4, rendant compte de situation où la mortalité est faible pour des échantillons de taille importante. Dans le cas général, on devrait donc vérifier que les modèles 2 ou 4 sont ceux qui représentent le mieux, l'évolution du nombre de morts quotidiens. La constante du modèle choisi est la variable qui sert à comparer dans une analyse de variance, les différents lots et les différents mutants.

2.5.3. La période 3 peut être caractérisée par le taux de mortalité en fin d'observation.

$$T_M = \left(1 - \frac{\text{N fin d'observation}}{\text{No}}\right) \times 100.$$

En effet, dès que la mortalité journalière tend vers 0, la taille de chaque lot se stabilise et le taux de mortalité représente le résultat de l'épreuve en fin d'observation. Ce taux de mortalité en fin d'observation est également une caractéristique de l'épreuve, si la mortalité journalière ne tend pas vers 0. C'est à dire, si en poursuivant l'observation, on observait un nombre de morts quotidiens non nul : il est le reflet de l'épreuve au jour j, choisi comme terme à l'observation quotidienne de la mortalité.

Le calcul de ce taux est complété par celui de l'index d'efficacité

$$IE = \frac{T_M \text{ Témoin} - T_M \text{ Essai}}{\text{TM Témoin}}$$

Le choix du témoin est important, car cet index caractérise la mortalité liée à l'essai. Il permet de normaliser les différents essais avec des témoins différents. Dans notre expérience, nous avons pris comme référence le seul témoin absolu.

3. RESULTATS

3.1. ETUDE DE LA MORTALITÉ LIÉE À L'IRRADIATION

Le tableau 1 donne les nombres d'animaux, vaccinés ou non, avant irradiation et le jour de l'épreuve. On peut constater quelque soit le mutant utilisé une mortalité non négligeable consécutive à l'irradiation. Cette mortalité a été étudiée grâce à une analyse de Variance (ANOVA) à deux facteurs controlés. Le premier facteur correspond aux « conditions d'irradiation » (irradiation de type I ou II, animaux vaccinés ou non) ; le deuxième facteur correspond à la « période d'observation » des effectifs de chaque lot : avant ou après irradiation. L'influence du facteur « type de mutant » n'est pas prise en compte. Le tableau ci-dessous donne les résultats de cette première analyse pour un risque de 1er espèce $\alpha = 5\%$. La valeur du F de SNEDECOR correspondante pour les degrés de libertés V_1 = degré de liberté du facteur étudié, V_2 = degré de liberté de la variance résiduelle est recherchée dans une table de F [9].

Tableau d'analyse de variance pour l'étude des effectifs des lots d'animaux vaccinés ou non avant et après irradiation

	Somme des carrés des écarts	Degrés de libertés	Carrés Moyens	F observé	Fα
Conditions d'irradiation	26.83	3	8.34	4.99	3.24
Période d'observation	42.67	1	42.67	23.82	4.49
Interaction	9.66	3	3.22	1.80	3.24
Variation résiduelle	28.66	16	1.79		

Nous pouvons remarquer une différence significative entre les effectifs des différents lots avant et après irradiation, et une influence significative de la vaccination sur la mortalité après irradiation. D'autres études des résultats du tableau 1 montrent qu'il n'y a pas de relation entre la période d'irradiation par rapport à la vaccination (irradiation de type I ou II) et la mortalité liée à l'irradiation.

Tableau 1

Répartition des lots d'animaux soumis à l'irradiation, avant irradiation et le jour de l'épreuve.
Etude de la mortalité des animaux vaccinés ou non en fonction du type d'irradiation

	Modalité d'irradiation I				Modalité d'irradiation II			
	Vaccinés et Irradiés		Irradiés		Vaccinés et irradiés		Irradiés	
Type de Mutant	Nbre d'animaux avant irradiation	Nbre d'animaux après irradiation j. de l'épreuve	Nbre d'animaux avant irradiation	Nbre d'animaux après irradiation j. de l'épreuve	Nbre d'animaux avant irradiation	Nbre d'animaux après irradiation j. de l'épreuve	Nbre d'animaux avant irradiation	Nbre d'animaux après irradiation j. de l'épreuve
Ra	21	18	21	18	22	21	22	22
Rc	21	17	23	21	20	16	23	21
Re	22	16	22	21	22	17	22	21

Tableau 2

Durée d'incubation de *Salmonella typhimurium* C_5S après épreuve

Mutant	Témoins	Témoins vaccinés	Vaccinés irradiés	Vaccinés non irradiés I	Vaccinés irradiés II	Vaccinés non irradiés II
Ra	4	4	7	4	4	4
Rc	3	5	5	5	8	8
Re	3	3	6	5	9	3

3.2. ETUDE DE LA DURÉE D'INCUBATION

Le tableau 2 rassemble les durées d'incubation de la maladie expérimentale et le tableau d'analyse de variance ci-dessous permet de comparer les différents « lots d'animaux » : Témoins, animaux vaccinés, animaux irradiés selon le protocole I et II animaux vaccinés et irradiés selon le protocole I et II, et les différents « mutants » utilisés.

Tableau d'analyse de variance pour l'étude de la durée d'incubation de la maladie expérimentale en fonction des différents lots d'animaux et du type de mutant

	Somme des carrés des écarts	Degrés de libertés	Carrés Moyens	F. Observés	F $\alpha = 5\%$
Lots d'animaux	36.61	5	6.82	3.43	3.33
Types de Mutants	0.4	2	0.2	0.11	
Variation résiduelle	19.56	10	1.956		

Nous pouvons constater des différences significatives entre les durées d'incubation de la maladie expérimentale, liées au traitements subis par les différents lots d'animaux. Par contre le type de mutant ne semble pas avoir d'influence sur cette incubation. Une étude similaire montrerait qu'il n'y a pas de différences significatives entre les durées d'incubation de la maladie expérimentale à l'intérieur du groupe d'animaux vaccinés (comparaison entre les animaux vaccinés, et vaccinés et irradiés). Le F observé est de 2.33 pour un F $\alpha = 5\%$ de 5.14. Par contre si l'on compare les lots d'animaux irradiés non vaccinés et les lots ni irradiés ni vaccinés, nous pouvons constater une différence significative limite pour un risque de 1 er espèce $\alpha = 5\%$: F observé = 5.33 pour F $\alpha = 5\% = 5{,}14$.

3.3. ÉTUDE DE LA MORTALITÉ JOURNALIÈRE

L'étude de l'ajustement des 4 modèles montre que le modèle le plus approprié est le modèle exponentiel. Nous avons donc dressé le tableau 3 qui donne deux valeurs extrêmes de la constante K_2 pour chaque lot et chaque mutant étudié. Cette constante K_2 est le taux de mortalité journalier moyen. Deux groupes de lots d'animaux se dégagent

— le groupe des animaux vaccinés

— le groupe des animaux non vaccinés.

La comparaison de ces deux groupes entre eux est inutile car le seul examen du tableau de résultats et des courbes de mortalités journalières permet de penser qu'ils sont significativement différents. Nous comparerons, à l'intérieur de chaque groupe, et pour chacune des expériences avec un mutant les taux de mortalité journalières. Pour ces comparaisons des analyses de variances ont été réalisées. Nous ne donnerons que les valeurs des F de Snedecor observés. Celle du Fα lu dans la table pour les nombres de degré de liberté (ddl) $V_1 = 2$ (ddl du facteur irradiation dans chaque groupe) et $V_2 = 3$ (ddl de la variation résiduelle) et pour un risque de première espèce $\alpha = 5\%$ est. F $\alpha = 5\% = 9.55$.

Tableau 3

Résultat de constantes de variation de la mortalité journalière K_2 définie par la relation $N = No\ e^{-K^2}\ NoJ$

		Témoins absolus	Témoins vaccinés	Irradiés vaccinés I	Irradiés non vaccinés I	Irradiés vaccinés II	Irradiés non vaccinés II
Ra	1	0,0062	0,0023	0,0027	0,0055	0,0024	0,0070
	2	0,0054	0,0019	0,0023	0,0049	0,0020	0,0060
Rc	1	0,0120	0,0021	0,0045	0,0060	0,0042	0,0052
	2	0,0095	0,0018	0,0036	0,0050	0,0032	0,0045
Re	1	0,0051	0,0023	0,0034	0,0092	—	0,0037
	2	0,0052	0,0018	0,0025	0,0077	0,0032	0,0038

3.3.1. *Mutant Ra*

— Groupe d'animaux non irradiés : F observé = 2.5

F observé $<$ F $\alpha = 5\%$

Il n'y a pas de différences significatives entre les mortalités journalières des animaux irradiés ou non irradiés.

— Groupe des animaux vaccinés ; F observé = 1.08

F observé $<$ F $\alpha = 5\%$

Il n'y a pas de différences significatives entre les mortalités journalières des animaux vaccinés, irradiés ou non.

Dans cette première expérience, l'irradiation ne modifie pas la mortalité journalière.

3.3.2. *Mutant Rc*

— Groupe des animaux non vaccinés : F observé = 16.2

F observé $>$ F $\alpha = 5\%$

Il y a une différence significative entre les mortalités journalières des animaux irradiés on non irradiés. Elle est diminuée après irradiation quelque soit le protocole I ou II.

— Groupe des animaux vaccinés : F observé = 7.9

F observé $<$ F $\alpha = 5\%$

Il n'existe pas de différences significatives entre les mortalités journalières après vaccination, les animaux ayant été ou non irradiés.

Dans cette seconde expérience l'irradiation modifie la mortalité journalière des lots d'animaux non vaccinés et non celle des lots vaccinés.

3.3.3 *Mutant Re*

— Groupe des animaux non vaccinés : F observé = 30 %

F observé $>$ F $\alpha = 5\%$.

Nous observons une différence significative, donc une influence du facteur « irradiation » sur la mortalité journalière. Le tableau 3 montre que celle-ci est augmentée après irradiation suivant le protocole I et diminuée après irradiation suivant le protocole II.

— Groupe des animaux vaccinés ; F observé = 4.2

F observé < F $\alpha = 5\,\%$

Il n'existe pas de différences significatives entre les mortalités journalières des lots d'animaux vaccinés qu'ils soient ou non irradiés.

Dans cette dernière expérience l'irradiation modifie également la mortalité journalière des lots d'animaux non vaccinés et non celle des lots d'animaux vaccinés.

Tabelul 4

Résultat des mortalités observées 20 jours après épreuve

		Témoins	Témoins vaccinés	Irradiés vaccinés I	Irradiés non vaccinés I	Irradiés vaccinés II	Irradiés non vaccinés II
Taux de mortalité	Ra	80 %	38,9 %	44,4 %	77,8 %	47,6 %	86,4 %
	Rc	85 %	38,9 %	43,8 %	71,4 %	37,5 %	81,8 %
	Re	85 %	23,5 %	23,1 %	90,5 %	13,3 %	76,2 %
Index de l'efficacité	Ra		0,51	0,44	0,03	0,40	0,0
	Rc		0,54	0,49	0,16	0,56	0,04
	Re		0,72	0,73	0	0,84	0,20

3.4. TAUX DE MORTALITÉ DE FIN D'ÉPREUVE ET INDEX D'EFFICACITÉ

Comme dans l'étude de la mortalité journalière on constate, sans avoir recours à une analyse statistique, une différence entre les lots d'animaux vaccinés d'une part, non vaccinés d'autre part. Nous avons deux groupes d'animaux ;

— groupe des vaccinés et vaccinés-irradiés.

— groupe des non vaccinés, irradiés ou non.

Dans chacun de ces deux groupes les intervalles de confiance des pourcentages de mortalité pour des tailles d'échantillon voisine de 20 montrent qu'il n'y a pas de différences significatives entre les lots d'animaux irradiés et non irradiés.

4. DISCUSSION

Les résultats des expériences effectuées pour étudier les effets d'une irradiation micro-onde à 2450 MHz sous 20 mW/cm² à raison de 4h/j pendant 4 jours montrent :

— qu'il existe une sensibilité plus grande, à l'irradiation, des animaux vaccinés par les mutants de *S. typhimurium* ;

— que l'irradiation selon le protocole I augmente de façon sensible la durée d'incubation de la maladie liée à l'administration du C_5S.

— que l'irradiation modifie de façon inconstante la mortalité journalière des animaux non vaccinés sans modifier celle des animaux vaccinés.

— que l'irradiation micro-onde ne modifie pas la mortalité globale liée à la maladie.

Ces quatre points méritent d'être discutés séparément. La sensibilité des animaux aux mutants de *S. typhimurium*, après irradiation est le premier point intéressant. En effet, il est possible de penser que cette sensibilité est liée à une modification du germe, à priori non pathogène, qui acquiert après irradiation une certaine pathogénicité. Cette hypothèse est possible, mais en contradiction avec d'autres observations [1], [11] qui montrent que l'irradiation microonde n'a pas de pouvoir mutagène. Une autre hypothèse, à notre avis plus vraisemblable, serait que l'irradiation microonde affaiblissant l'organisme animal, le rendrait plus sensible à la pathogénicité latente des mutants. En effet on sait que ces mutants ne sont vaccinants que s'ils sont injectés au-dessous d'un certain seuil de concentration [6], [7]. Au dessus, leur nombre est tel qu'ils deviennent toxiques. Il est tout à fait possible que l'irradiation microonde puisse abaisser le seuil de sensibilité d'un organisme à un germe pathogène ou subpathogène.

Le deuxième point porte sur les durées d'incubations de la maladie expérimentale. Nous remarquerons que la durée moyenne de cette période est de 3 à 5 jours pour une injection de 5×10^5 germes environ. Elle n'est allongée que dans les lots d'animaux irradiés selon le protocole I ou vaccinés et irradiés selon les protocoles I et II. L'irradiation et plus particulièrement lorsque l'on utilise le protocole I pourrait agir sur certain processus de défense d'expression retardée (défense immunitaire non spécifiques par exemple) retardant sans toutefois l'empêcher, l'expression de la pathogénicité du germe d'épreuve.

La modification inconstante de la mortalité journalière est également intéressante à ce point de vue. Dans la majorité des cas on observe une modification dans le sens d'une réduction faible mais sensible de cette mortalité. Cette modification, comme dans le point précédent peut être envisagée sous l'angle d'une modification inconstante mais sensible des systèmes de défense non spécifiques.

En conclusion il existe une certaine conjonction d'observations qui nous amènent à penser que l'irradiation micro-onde n'est pas dénuée d'effet sur les systèmes de défense de l'organisme. Ces effets peuvent être envisagés sous 2 aspects :

— un effet immédiat de type stress conduisant à un affaiblissement passager d'un organisme, le rendant plus sensible à un agent pathogène ou subpathogène.

En effet plus ou moins retardé se manifestant par des modifications, le plus souvent une stimulation, des systèmes de défense non spécifiques. Cette modification est de faible amplitude et relativement fugace, ne conduisant pas à une protection globale des individus ou n'amplifiant pas la protection liée à une vaccination par un germe vivant. Cette stimulation de processus immunitaire, non spécifique est en accord avec des observations déjà faites précédemment [5], [8], [10], sur des tests explorant l'immunité cellulaire, effectués après irradiation microonde, en cavité multimode ou anéchoïque [3]. Pour explorer de façon plus

complète l'impact des microondes sur le système immunitaire de la souris il serait nécessaire d'augmenter le temps d'irradiation et peut être de faire des études permettant de conjuguer différentes longueurs d'onde.

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DISACILIN MEMBRANE EFFECTS

BY

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The influence of disacilin upon striated frog muscle fibres membrane potential has been followed up, as well as the distribution through the membrane of sodium, potassium, and calcium ions on the one hand and water on the other, between the intracellular and extracellular mediums in rabbit muscular, hepatic, and intestinal tissues. The biostimulator has been found to influence membrane potential and permeability, determining an intensification of ion active transport and a water and ionic equilibrium which favours the evolution of metabolic processes.

A growth biostimulator can achieve its specific effects at different levels in the organism also by direct action on cell membranes. Through this type of actions it can influence membrane permeability to ions and water, which results in the modification of ion ratios in the intracellular and extracellular mediums. Both membrane potential and the transport of amino acids, sugars, and lipids through the membrane [4], [12] may be modified in this way, and cellular metabolism too, since there is a close connection between them [10].

In order to evidence the direct effects upon cell membranes determined by disacilin, a product with properties of growth biostimulator [1], [6], we have followed up its action upon membrane potential and water and ion distribution between the intracellular and extracellular compartments in different tissues.

MATERIAL AND METHODS

Disacilin influence upon membrane potential has been followed up in the case of the frog sartorius striated fibres membrane by glass intracellular microelectrodes. Ringer solutions with bicarbonate as a buffer (pH = 7.2) were administered in a continuous flow at the room temperature. Disacilin has been used under the form of tartrate in concentrations of 0.1 mM. In order to observe its influence upon the active transport of ions through the membrane, ouabain, in a concentration of $5 \times \times 10^{-6}$ M, was used as a blocking of $Na^+ - K^+$ pump. The statistic evaluation of results has been made by the Student method.

Disacilin influence upon water and sodium, potassium, and calcium ions distribution in the intra and extracellular mediums has been studied in tissues drawn from the muscles, liver, and small intestines of three mature laboratory rabbits. From each rabbit two series of samples (ca. 1 g of tissue each) were taken and subsequently incubated in saline. For a

control series normal saline was used. For the other, saline containing disacilin in a concentration of 20 mg/ml was used.

Total water evaluation was made by differentiating the weight of the fresh tissue ant that of the same tissue after having been dried at 105 °C in the drying closet. Extracellular water was measured by the method of the insulinic space and the intracellular one by calculation. The average values were expressed in ml/100 g fresh tissues.

The evaluation of the total amount of sodium, potassium and calcium was made by the flame-photometric methods and the distribution of ions between the intra and extracellular medium was evaluated by comparison with the intra and extracellular space. In control samples the average results were expressed in mg /100 ml total water, either intra or extracellular ; and in the treated tissues in percentage as compared to control samples.

RESULTS

As regards disacilin influence upon membrane potential we have found out that it determines, in a concentration of 1 mM, an effect of intense depolarization and a tendency of lysis of the cell membrane. That is why a concentration of only 0.1 mM was chosen. For this concentration the product determines only a slight and unstable membrane hyperpolarization (Fig. 1) with a clear tendency of disappearing in the course of time.

If the activity of $Na^+—K^+$ pump is blocked with ouabain (Fig. 2), disacilin 0.1 mM does not achieve its specific hyperpolarization effect. There can be pointed out only a depolarizing stable action upon the membrane, with an average amplitude of 5.23 mV, slightly reversible when reintroducing the fibres in normal Ringer. This proves that in ordinary conditions disacilin specific effect upon the membrane comes out of two different actions, one of these being achieved on the basis of an interference with the active transport of ions.

The results concerning the influence of disacilin upon the distribution of water and ions between intra and extracellular mediums in different tissues support this conclusion.

As regards water distribution in muscles, liver and the intestinal wall (Fig. 3) disacilin seems to have a small influence upon total water. A slight increase of the quantity of total water in muscles (with 2.80 %) as compared to a control sample and a slight decrease of it in the small intestine (with 3.0 %) can be noticed. The increase ascertained in muscles is achieved mainly by the increase in the intracellular quantity of water, and the decrease in the intestinal tissue is due to the decrease of intracellular water in principle. The distribution of water in the hepatic tissue is more strongly influenced by disacilin. Total water remains the same but

the amount of extracellular water increases very much (with 51.27 %), while the amount of intracellular water decreases (with 25.03 %).

The distribution of ions in the studied tissues is nevertheless strongly influenced by disacilin. The total concentration of sodium (Fig. 4) is slightly increased in muscles (with 14.77 %) and liver (with 12.80 %) and almost unchanged in the intestine. Disacilin action is more evident as regards

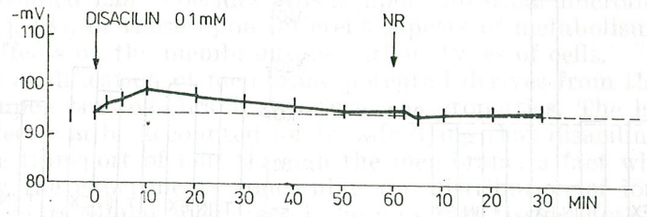

Fig. 1. — The effect of disacilin on the membrane potential in normal conditions.

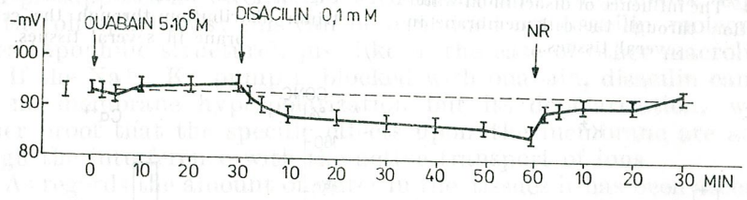

Fig. 2. — The effect of disacilin on the membrane potential when active transport was blocked.

the distribution of sodium between the intra and extracellular mediums. The increase in the total concentration of sodium in muscles is achieved by the increase of extracellular concentration, the intracellular one being low. The same phenomenon can be observed in the intestinal tissue, too, while in the liver an increase in the concentration of sodium both in the extra and intracellular compartments is noticed. This supposes an intensification of the active transport of sodium.

As regards the distribution of potassium, disacilin determines more important modifications as compared to control samples (Fig. 5). The total concentration of potassium increases very much in the muscular and hepatic tissues (with 76.50 % and 20.55 %, respectively) and decreases in the intestinal one (with 22.27 %). The increase observed in muscles and liver is achieved by an exogenous contribution, since both the intra and the extracellular concentrations increase ; the increase of the intracellular concentration is evident. This means that the transport of potassium through the membrane is intensified under the influence of disacilin ; the increase in the active transport is more evident than that of the passive one. However, in the case of the intestine the intracellular concentration decreases and the extracellular one increases. This comes out of the intensification of the passive transport of potassium.

The distribution of calcium presents similar aspects (Fig. 6). Under the influence of disacilin, the total concentration of calcium increases in muscles (with 36.15 %) and in the liver (with 26.0 %) and decreases in the intestinal wall (with 41.48 %). These modifications are achieved almost

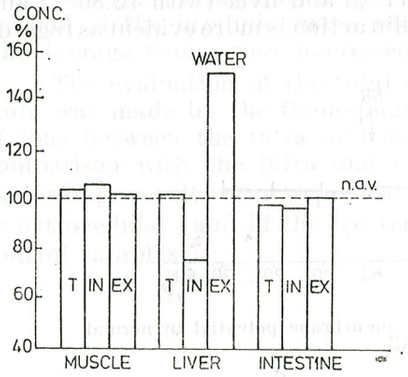

Fig. 3. — The influence of disacilin on water distribution through the cell membrane in several tissues.

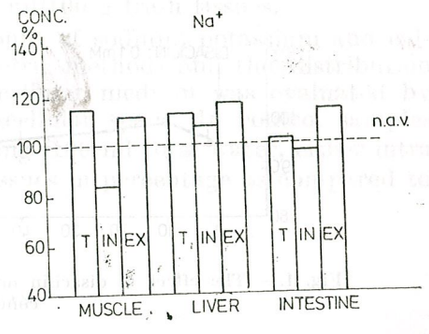

Fig. 4. — The influence of disacilin on natrium distribution through the cell membrane in several tissues.

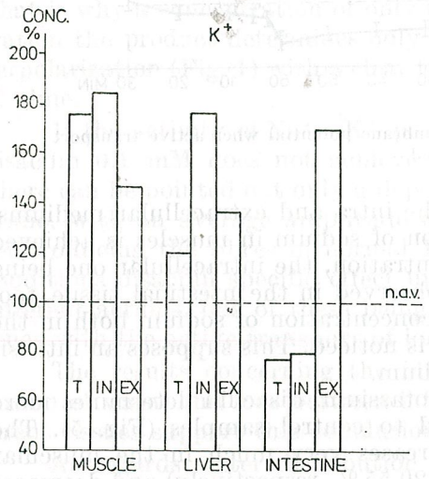

Fig. 5. — The influence of disacilin on potassium distribution through the cell membrane in several tissues.

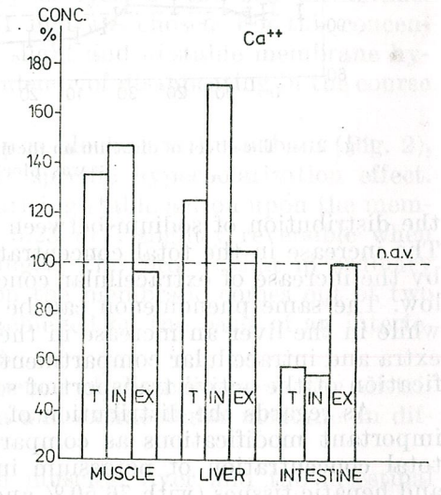

Fig. 6. — The influence of disacilin on calcium distribution through the cell membrane in several tissues.

exclusively through the modifications of intracellular calcium concentration, which increases in muscles and in the liver and decreases in the intestine. In the case of muscles and of the liver, disacilin determines an inhibition of the active transport of calcium and in the case of the intestine an intesification of the active transport or a reduction of the passive per-

meability of the membrane for calcium. The increase of the intracellular potassium and calcium concentrations in the liver can be due to a great extent to the decrease of intracellular water in this tissue.

Discussions and conclusions

From the results obtained we can conclude that the growth biostimulator studied has — besides effects upon intestinal microflora already known [1], [8], or those upon different aspects of metabolism [3] — important effects on the membranes of various types of cells.

The modification of membrane potential derives from the action of disacilin upon the membrane structure and properties. The hyperpolarization effect can be accounted for by admitting that disacilin stimulates the active transport of ions through the membrane, a fact which is confirmed by the experiments concerning the distribution of ions between intra and extracellular mediums of the muscular tissue, both for sodium and for potassium. At the same time, a reduction of the membrane passive permeability to sodium takes place. In a concentration of 1 mM, disacilin produces a depolarization with a tendency towards membrane lysis, which presupposes an interaction with membrane components, especially with the phospholipids; this can be explained by disacilin molecule properties (lipophilic structure), just like in the case of other macrolids [5].

If the $Na^+ — K^+$ pump is blocked with ouabain, disacilin can determine no membrane hyperpolarization but its depolarization, which is another proof that the specific effects upon the membrane are achieved through the interference with the active transport of ions.

As regards the amount of water in the tissues it has been ascertained that disacilin does not induce a significant growth of the total amount of water. The increase in weight of the animals treated with disacilin is not due to the hydration of the tissues. At the same time, the maintenance of an increased amount of intracellular water can be observed (with the exception of the liver), a fact which represents a positive effect if we consider that with the age the total tissular water of animals decreases especially through the decrease of the intracellular one [7], [11].

The total amount of sodium is generally increased in comparison to the reference values and this is a positive fact considering the decrease of the quantity of sodium with the age [11]. Disacilin influence upon the distribution of potassium in the tissues is more evident. The important increase of total and intracellular concentrations in muscles and liver can be correlated with the increase in the amount of glycogen and a modification of protein synthesis [1], as well as the decrease in intracellular water. Some authors [11] have shown that potassium decreases very much in underfed animals.

As for calcium, it can be admitted that in certain tissues its total amount decreases with the age [9], [11]. Our experiments proved that disacilin reduces an increase in calcium concentration especially in muscles and liver. We can consider this effect, too, as being positive with respect to the biostimulative action.

From all our experiments, disacilin has been proved to induce an intensification of ions transport through the membrane (especially the

active transport). This presupposes an intensification of energy productive metabolic processes correlated with this transport. Through its action, disacilin determines a new value of intra and extracellular ions concentrations ratios, which is similar to the one characteristic of younger animals. The modifications induced are compatible with the evolution of cell and membrane processes within normal limits. They are the expression of the establishment of a new physiologic equilibrium. From its effects at the membrane and cell level comes out a greater plasticity of the different processes taking place therein a fact which can be considered favourable for the use of disacilin as a biostimulator of animal growth.

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THYMO-BURSAL REACTIONS TO LEVAMISOLE IN CHICKENS

BY

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Studler-Cornish chickens aged 5 days were injected a single i.p. dosis of levamisole (0.25 mg per animal). Modifications were followed in the thymus (T), bursa of Fabricius (B), adrenals (A), and blood serum (S) 8 hours, and 1, 3 and 7 days after drug administration.
B was more heavily affected than T.A gain in weight and in nucleic acids content were noticed in B even at 8 h after injection. A loss of weight and an increase of the glycogen content (i.e. involution phenomena) were only noticed in T, beginning at least 3 days after the treatment; nucleic acids showed no modification. Total proteins of T and B, ascorbic acid of A, total protein and gammaglobulin content of S did not show modifications; glycogen content of A was increased.
The modifications observed in B may lead to a stimulation of the humoral immunity *via* the bursa-dependent cells.

Effects of levamisole on avian [15], mammalian [14] and human [13] organisms have been much investigated during the last years. Stimulating effects have been put into evidence on the immunological reactivity. *In vitro* studies on lymph cell cultures have shown a stimulation of the proliferation [6], [7], [10], [11].

No data are available concerning *in vivo* biochemical effects of levamisole upon the thymus and the bursa of Fabricius of the chicken. The aim of the present paper is to furnish such data for a developmental stage when the immunobiological processes are still organizing.

MATERIAL AND METHODS

Studler-Cornish (Robro-69) chickens were injected when 5 days old with a unique i.p. dose of levamisole (Richter Gedeon, Hungary), 0.25 mg per animal. Control chickens received a similar volume of physiological saline (0.1 ml/animal). All animals were maintained in conditions similar to those of poultry farms, fed on a concentrated fodder and water *ad libitum*. Eight chickens per group were sacrificed by decapitation 8 hours, and 1, 3 and 7 days after the administration of the drug. The following parameters were determined: the weights of T and B; total protein by the biuret reaction [4], in blood serum, T and B; gammaglobulin content of blood serum, by specific $(NH_4)_2SO_4$ precipitation and biuret reaction [19]; glycogen content of T, B, and left adrenal, by the phenol-sulphuric reaction after KOH dissolution of the tissue [8]; total ascorbic acid content of the right adrenal, by a photocolorimetric method using the di-

nitrophenylhydrazin reaction [5]; RNA and DNA content of T and B, by differential spectrophotometry of perchloric tissue extracts [16].

Statistical processing of the data involved elimination of aberrant individual values according to Chauvenet's criterion and checking of differences between treated and control groups by Student's "t" test.

RESULTS

Modifications in the thymus and bursa of Fabricius (Table 1)

Total protein concentration is not modified in the lymph organs, and at any time interval after drug administration.

Table 1

Total protein (TP), RNA, DNA and glycogen (G) content in bursa of Fabricius and thymus, and organ weight (TW and BW) after levamisole administration in chickens. Values are mean ± standard errors

Sacrification		8 hours	1 day	3 days	7 days
			Thymus		
Control					
TP	(mg/g)	355.8±107.5	355.8±107.5	303.4±3.68	259.6±10.1
RNA	(mg/g)	2.1± 0.4	2.1± 0.4	3.3±0.4	3.3± 0.4
DNA	(mg/g)	3.2± 1.0	3.2± 1.0	4.8±1.1	3.4± 0.7
G	(mg/g)	0.6± 0.1	0.6± 0.1	1.0±0.2	1.0± 0.1
TW	(mg)	80.0± 15.0	80.0± 15.0	53.1±6.1	83.7±18.0
Treated					
TP		283.4±84.0	237.4± 22.0	321.2±27.8	282.8±13.9
RNA		2.2± 0.2	2.8± 0.9	3.5± 0.7	3.1± 0.3
DNA		2.5± 0.3	2.2± 0.7	6.8± 1.5	4.3± 0.6
G		1.0± 0.2	0.9± 0.1	1.6± 0.2	1.2± 0.2
TW		124.2± 9.9	62.6± 7.7	32.1± 5.4	44.5± 5.1
			Bursa of Fabricius		
Control					
TP		243.2±51.6	243.2± 51.6	256.5± 20.6	182.4±31.3
RNA		1.6± 0.3	1.6± 0.3	3.2± 0.9	3.5± 0.2
DNA		2.9± 0.2	2.9± 0.2	7.6± 2.8	3.5± 0.3
G		0.8± 0.1	0.8± 0.1	0.5± 0.06	0.5± 0.03
BW		51.0± 5.1	51.0± 5.1	35.3± 3.6	65.1± 6.2
Treated					
TP		357.6±76.1	203.8± 10.5	281.8± 26.0	236.3±22.5
RNA		2.8± 0.2	4.9± 0.6	4.4± 1.5	3.5± 0.6
DNA		2.9± 0.4	3.9± 0.3	6.3± 0.9	3.5± 0.7
G		0.7± 0.08	0.6± 0.1	1.1± 0.3	1.3± 0.1
BW		73.7± 6.4	56.6± 7.3	46.2± 1.8	30.8± 2.7

RNA of the bursa of Fabricius exhibits an 80 % increase after 8 hours ($p < 0.01$) and a 210 % one after one day ($p < 0.001$), as compared to the values of control animals.

DNA increases somewhat later: +34 % ($p < 0.01$) in B one day after the injection of levamisole.

Glycogen content on unit tissue weight increases in both lymph organs beginning with the third day after drug administration. In T, by +57 % ($p < 0.01$) after three days, the value falls back to that of the controls after 7 days. On the contrary, in B the increase (98 %, $p < 0.01$) by the 3rd day is enhanced as time proceeds: 152 % ($p < 0.001$) by the 7th day.

Weights of the lymph organs increase after levamisole administration: +55 % ($p < 0.02$) for T and +44 % ($p < 0.01$) for B, at 8 hours. By the third day, a 40 % decrease ($p < 0.01$) is noticed in T, while B maintains a 30 % ($p < 0.01$) increase. By the 7th day both organs exhibit decreases: −47 % ($p < 0.01$), respectively −53 % ($p < 0.001$).

Modifications in the adrenals and blood serum (table 2)

No significant modifications are found in the adrenal *ascorbic acid* content, as compared to the corresponding control values in any of the studied post-administration periods.

Table 2

Modifications in the adrenals (ascorbic acid — A, and glycogen — G) and in blood serum (total protein — TP, and gammaglobulin — Gg) after levamisole administration in chickens. Values are mean ± standard errors

Sacrification		8 hours	1 day	3 days	7 days
			Adrenals		
Control					
A	(μg/mg)	3.08±0.26	3.08±0.26	3.26±0.11	2.32±0.15
G	(mg/g)	2.09±0.22	2.09±0.22	0.93±0.20	0.68±0.07
Treated					
A		3.08±0.20	2.86±0.31	3.47±0.44	2.85±0.20
G		3.01±0.82	0.88±0.12	1.07±0.14	1.32±0.18
			Blood serum		
Control					
TP	(mg/cm³)	28.70±0.97	28.70±0.97	27.68±2.98	37.15±2.03
Gg	(mg/cm³)	7.47±0.23	7.47±0.23	7.01±0.61	6.47±0.52
Treated					
TP		29.87±0.84	27.87±1.27	27.35±2.0	32.52±2.56
Gg		7.21±0.41	7.23±0.51	7.30±0.54	6.52±0.27

Glycogen content of the adrenals is lowered by 58 % ($p < 0.01$) one day after injection, and increased by 94 % ($p < 0.01$) after 7 days.

Serum protein and *gammaglobulin* content do not show any significant modifications.

DISCUSSION

A single dose of levamisole, administered to young chickens in a developmental stage in which the immunobiological processes are not yet fully organized, induces large modifications in the central lymph organs,

especially in B, beginning with the first few hours after injection. The effects on B are of stimulation : increases of the weight and of the content of nucleic acids. They possibly indicate a stimulation of B-dependent elements, and thus an enhancement of the processes connected with the humoral immunity. Soppy et al. [15] have shown *in vivo* stimulating effects of levamisole upon cellular and humoral immunity ; the T cells would be activated, the drug acting as an immunological adjuvant of the latter, modulating this way the immune reaction. On the other hand, Symoens [18] argues that levamisole action is not properly an immunitary stimulation, but a normalization of the function of lymphocytes and phagocyting cells when the activity of these immune elements had been depressed. Our data show more pronounced effects on the bursa than the thymus ; as it is known that the functional importance of both organs varies during the ontogenetic development, it is possible that our observation may be determined by the given developmental stage (and also by the dose used). Indeed, it has been shown that the effects of levamisole on lymph cells are dependent on their stage of activation [9].

The early increase of RNA content of B may be attributed to an inhibition of the lysosomal enzymes and an activation of RNA -polymerase [2], [3]. An enhancement of DNA synthesis has been shown *in vitro*, in experiments on thymus or spleen cells [7], [20]. Our results suggest also that the drug acts directly on the lymph organs ; an adrenal-mediated action is not likely, as no modification was noticed in the ascorbic acid content of this gland. Only in later stages (3 and 7 days after drug administration) stress-induced modifications [12] seem to occur in T and B : a ponderal involution and an increase of the glycogen content (but, in our case, without any decrease of the nucleic acids). However, even in this stage, we cannot suppose a hypersecretion of glucocorticoid hormones, for which the lymph organs possess receptors [1], [17], as the ascorbic acid content of the adrenals is not modified.

Otterness et al. [10] have shown two interconnected actions of levamisole in cell cultures : an increase of the number of proliferating cells, and an enhancement of the cell cycle rate of these. This finding supports also our assumption that levamisole affects directly the lymph cells, not *via* an endocrine mediation.

In conclusion, levamisole administered in a single dosis to young chickens has important stimulating effects upon the central lymph organs, especially B. The action seems to be direct and not adrenal-mediated.

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PRESENCE OF ARYLSULPHATASE ACTIVITY WITH INSECTS

BY

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Arylsulphatase activity with insects was measured, without relating its ratio between A and B arylsulphatases. Experiments generally considered the body as a whole, except certain tests performed separately on both intestine and the rest of the body. Most of the analysed insects proved highly arylsulphatasic.

Arylsulphatases are ubiquitarian enzymes, found with bacteria, fungi, mollusca, birds and mammals [2], [3]. Mammalian liver, kidney, adrenal gland [2], testis [9] and seminal plasma [5] are rich in arylsulphatases. The physiological rôle of these enzymes was lately established : the natural substrata of A arylsulphatase are made of cerebroside 3-sulphate, seminolipid and ascorbin 2-sulphate, while UDP-N-acetylgalactosamine 4-sulphate [4], [9] characterizes arylsulphatase B. Both A and B arylsulphatases are glycoproteins found in lisosomes. Little is known about arylsulphatases and insects. The present paper deals with entomological arylsulphatase activity.

MATERIALS AND METHODS

Materials. The synthesis of 4-nitrocatecol sulphate was shown in a previous paper [5]. The insects were collected around Bucharest and Lake Herăstrău, and were afterwards vivisected.

Measurement of the arylsulphatase activity. The insects were homogenized and extracted at 1 : 20 distilled water, for 2h at 4 °C. The homogenate was centrifugated for 10 min at 4,000 g, preserving the supernatant. An amended Worwood et al. method [8] was used to establish arylsulphatase activity : 0.5 ml enzyme sol mixed with 0.5 ml nitrocatecol sulphate sol (NCS), using a 20 mM buffer acetate 0.5 M pH 5.5, incubated for 4 h at 37 °C. The enzyme activity was stopped by adding 1.5 ml sol NaOH 0.5 N. The absorption of the issued nitrocatecol was measured at 515 nm. Enzyme activity was determined according to [5].

Protein determination assay. The Lowry et al method [7] was used, checked with bovine serum albumine as standard.

RESULTS AND DISCUSSIONS

Arylsulphatase activity with different insects is shown in Table 1. Most of these organisms have a similar arylsulphatase activity with that of the chicken brain homogenate [3] and boar seminal plasma [5].

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Table 1

Arylsulphatasic activity in some insects

Name of insect	No. of individuals	Enzymic activity nmol/mg/h
Scotia segetum (larva)	2	166
Eurydema ornata	2	124
Pyrrochoris apterus L.	3	114
Coccinella quatrodecem pustulata	2	111
Gryllus campestris (intestine)	1	57
Eurigaster intergriceps	6	96
Abax paralellus Duft	1	90
Coccinella septempunctata L (intestine)	7	78
Locusta migratoria (intestine)	1	74
Ophonus rufipes Deg	1	48
Anisodactilus signatus	1	48
Lymantria dispar (larva) (intestine)	1	33
Dermestes erichsoni	2	29
Amara crenata	1	27
Clytra quadripunctata	5	20
Dorcus paralellopipedus	1	24
Bombus hortorum	1	9
Eristalis	1	1.2

However, the presence of arylsulphatase activity inhibitors is not excluded with insects, as proved with chicken brain [3] and *M. gallo-provincialis* — whole body [6]. Besides, the real enzymatic activity can be greater than we found if optimal circumstances are approached. Considering the comparatively long time of incubation, the influence of nitrate reductases — very active with some organisms — could also diminish the real value of arylsulphatase activity by conversing the nitrophenol of that very amine [2]. Even if enzyme activity proves practically null — e.g. with *Bombus hortorum* and *Eristalis* — , the arylsulphatase activity assay must be reconsidered since nitrocatecol sulphate is used as substratum. Most insects proved arylsulphatasically active, certain intestines too. Nevertheless, a more accurate siting of the nervous system, alimentary tract and reproductory functions is required together with a quantitative ration between different types of arylsulphatases, the more so as Worwood et al. [8] showed that mixed A and B arylsulphatase dosage cannot always be done if Baum and Dodgson's method [1] is used, a previous separation being therefore compulsory.

The study of arylsulphatase and organic sulphate with insects is also interesting for a more comprehensive understanding of their physiological rôle. If further considering enzyme relation with the biological cycle of insects, a lot of new outstanding data can be revealed on their metabolism.

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THE CORRELATION BETWEEN FILTERING ZOOPLANKTON AND PHYTOPLANKTON. III. THE ENERGY EXPENDITURE AS A FUNCTION OF FOOD CONCENTRATION, TEMPERATURE AND PARTIAL OXYGEN PRESSURE

BY

ANGHELUȚĂ VĂDINEANU and RALUCA MUNTEAN

The implications of varying food concentrations, changing temperature and PO_2 upon energy expenditure of *Daphnia magna*, *D. pulex*, *Simocephalus vetulus* (Cladocera) and *Eudiaptomus gracilis* (Copepoda) are reported. The established mathematical models, the multiple correlation coefficient and "F" test values are presented also.

Since filtering crustaceans have a differentiated filtering apparatus with a role both in food collecting and in gas exchange, it has been considered that filtration rate maintains itself to a minimum value to ensure the necessary oxygen for an increase in the food concentration above 15—20 mg dry weight/l [6], [13], [4].

Studies carried out on *D. magna*, *D. pulex*, *Simocephalus vetulus* (Cladocera) and *Eudiaptomus gracilis* (Copepoda) grown in media with food concentration higher than 20 mg dry wt/l have shown that filtration rate does not stabilize around a minimal value, but still decreases as the algae concentration increases [14], [15].

The increasing concentration of food in suspension above a certain limit (15—20 mg dry wt/l) and the associated reduction in volume of filtered water induces conditions of oxygen deficiency. This in turn has an important effect on the energy flow. The natural populations of filtering Cladocera and Copepoda of the fresh water ecosystems with specific levels of food resources can be in various equilibrium states.

Since we consider the change from one state to another to be promoted by a number of associated factors of which at a given moment only some have a determinant role we have looked at the implications of varying food concentrations upon energy expenditure in close connection to changing temperature and partial oxygen pressure (PO_2).

MATERIALS AND METHODS

The work has been carried out on laboratory models with three species of Cladocera: *Daphnia magna*, *D. pulex* and *Simocephalus vetulus* and by a species of Copepoda, *Eudiaptomus gracilis*. As a food source the

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green algae — Chlorella and Scenedesmus — were used. The main environmental factors, food concentration, temperature and PO_2 were varied within the usual limits found for the oligotrophic and highly eutrophic waters. The algae concentration, as determined at 670 nm, ranged between 0.3 and 50 mg dry wt/l, the temperature varied from 15 to 25° C and PO_2 had values between 15 and 160 mmHg. The Cladocera used were between 3 and 70 μg dry wt. and the Copepods between 1 and 25 μg dry wt. Samples were dried for 48 hours at 60 °C and weighed on an electrical balance with 1 μg sensivity. For a period of 2—5 days groups of similar size animals were acclimated to the experimental conditions. The energy expenditure expressed as oxygen consumption has been determined with a Combi analyzer equipped with type Ea 1 : Ac microelectrodes and by establishing the activity of the electron transporting system (ETS) by the Packard method (1971).

A value of 3.9 has been used for the conversion factor F in order to achieve correct values in μl O_2/h for absorption at 480 nm [8]. Finally, oxygen consumption has been expressed in μ$l0_2$/mg dry wt/h according to the formula :

$$R_1 = \frac{3\,H\,F\,A^{480}}{\text{mg dry wt of sample}}$$

where :

— 3 is a constant which gives the possibility to express activity per hour,

— H is the volume of homogenate obtained for each sample.

Oxygen consumption has been expressed in energy units using values of 3.38 cal/mg O_2 and 4.83 cal/ml O_2 for the oxicaloric coefficient [3].

The energy expenditure via the anaerobic pathway (R_2) has been determined by measuring the rate of lactic acid built up [1]. Knowing that from one milligram of glucose whose energetic values is 3.8 cal [16], [9], one milligram of lactic acid is obtained as final product, the quantity of accumulated lactic acid was expressed in energy units.

RESULTS AND DISCUSSIONS

According to the range of fluctuation of the parameters considered the energy expenditure data were grouped so as to define four distinct states :

(1) Concentrations of available food below 1—2 mg dry wt/l and PO_2 above 100 mm Hg. Under this set of conditions the energy requirement for maintenance is released from the substrate wholly by aerobic processes. The oxygen consumption in this case depends on the size structure of the population and temperature. Obviously the possible effect of other chemical factors on the metabolic processes is not excluded and their effects can be taken into account accordingly by the mathematical model.

(2) Food concentration between 1 and 5 mg dry wt/l but PO_2 falls below 40 mm Hg. Under these conditions the oxygen consumption rate depends on the size of the individuals, temperature and PO_2. Although this particular combination of parameters is unlikely to occur in natural

ecosystems, it was included in our experiment in order to obtain additional information on the response mechanism of these populations.

(3) Food concentration varies widely (1—50 mg dry wt/l) and PO_2 exceeds 100 mm Hg. Energy expenditure for maintenance is expressed as oxygen consumption depending on individual size, food concentration and temperature, as well as energy expenditure via the anaerobic pathway depending on the same factors.

Table 1

Energy expenditure as a function of food concentration (H), temperature (T), partial oxygen pressure (PO_2) and individual size (W) for *Daphnia magna*, *D. pulex* and *Simocephalus vetulus*

(R_1 — mg O_2/mg dry wt/h ; R — cal/mg dry wt/h ; H — 20 mg dry wt/l ; PO_2 — mmHg ; W — mg dry wt)

No	Y	X	Mathematical model	R^2	F
1	R_1	W, T	$R_1 = 0.0007e^{0.083T}W^{-0.43}$	0.87	37**
2	R_1	W, H, T	$R_1 = 0.0007e^{(0.083T-0.012H)}W^{-0.43}$	0.92	28**
3	R_1	W, T, PO_2	$R_1 = 0.00013e^{0.097T}W^{-0.415}PO_2^{0.4}$	0.85	21**
4	R_1	W, H, T, PO_2	$R_1 = 0.00013e^{(0.097-0.01H)}W^{-0.415}PO_2^{0.3}$	0.9	45**
5	R_2	W, H, T	$R_2 = 0.0001e^{(0.091T+0.045H)}W^{-0.39}$	0.83	17**
6	R_2	W, H, T, PO_2	$R_2 = 0.00062e^{(0.082+0.045H)}W^{-0.39}PO_2^{-0.41}$	0.88	23**

** — significant for P = 0.01.

Table 2

Energy expenditure as a function of food concentration (H), temperature (T) and individual size (W) for *Eudiaptomus gracilis*

(R_1 — mgO_2/mg dry wt/h ; R_2 — cal/mg dry wt/h ; H — 20 mg dry wt/l)

No	Y	X	Mathematical model	R^2	F
1	R_1	W, T	$R_1 = 0.00067\ e^{0.084}W^{-0.393}$	0.94	23**
2	R_1	W, H, T	$R_1 = 0.00067\ e^{(0.084T-0.015H)}W^{-0.39}$	0.92	15**
3	R_2	W, H, T	$R_2 = 0.000024\ e^{(0.088T+0.049H)}W^{-0.468}$	0.88	19**

** significant for P = 0.01.

(4) In this case food concentration fluctuates between 10 and 50 mg dry wt/l and oxygen concentration becomes critically low. The energy expenditure in this case is expressed both by oxygen consumption and by lactic acid built up and is dependent on temperature, individual size, food concentration and PO_2.

The experimental results have been quantitatively analyzed and multiple regression models have been established for the Cladocera and *Eudiaptomus* species respectively, expressing energy expenditure via aerobic and anaerobic pathways.

The established mathematical models, the multiple correlation coefficient and F test values are presented in tables 1 and 2.

It can be noted that values determined for the dependent variable are 80% justified by the independent variables considered. The energy ex-

penditure expressed as oxygen consumption for the species of Cladocera and Copepoda studied, under nonlimiting food availability and PO_2, is rather widely varying with temperature and body size. Thus, at 20 °C the oxygen consumption of Cladocera of 8 to 70 μg dry wt, reached values of 6 to 19 μl, whilst Copepods of 3 to 20 μg dry wt, under the same experimental conditions, used up between 2.5 and 8 μl O_2/24 h/individual. The experiments at 10 °C for the same body size classes have shown oxygen consumption rates of 2.6—8 μl O_2/24h/individual for Cladocera and of 1—3.5 μl0_2/24h/individual for Eudiaptomus. Based on these results the value of coefficient Q_{10} is 2.3 for all species studied. La Row et al. [7] report a Q_{10} coefficient of 1.75 for *Daphnia galeata* and *Diaptomus siciloides*. The oxygen consumption rates reported in lieterature generally fall within close range of the values reported in the present paper. For *Daphnia pulex* oxygen consumption rates reached values of 2—12 μl O_2/24h/individual [13].

Based on results regarding the dependence of filtration rate on excessive food concentration it was deduced that above 20 mg dry wt/l of food, conditions of hypoxia will be induced indirectly.

The assumption that oxygen consumption rate would be limited by increasing food concentration was proven by our results. With individuals of Cladocera species averaging 8 μg dry wt, at 20 °C and food concentration below 20 mg dry wt/l, the oxygen consumption rate was 29.5 μl O_2/mg dry wt/h whereas at a food concentration of 50 mg dry wt/l, the specific oxygen consumption decreased to 16.2 μl O_2/mg dry wt/h which means a 44 % reduction in oxygen consumption.

The specific oxygen consumption also showed a marked decrease with increasing food concentration when the experiments were carried out at different temperatures and with individuals of different size groups. For *Eudiaptomus gracilis* of an average of 8 μg dry wt/ind. kept at 20°C, a specific oxygen consumption of 25 μl O_2/mg dry wt/h was recorded when food concentration was below 20 mg dry wt/l. A 53 % decrease in oxygen consumption down to 11.9 μl O_2/mg dry wt/h was recorded for the same experimental set up when food concentration reached 50 mg dry wt/l. Under these conditions of hypoxia induced by reduction of filtration rate due to excess food, the individuals used partially the anaerobic pathway in order to get up the energy for maintenance.

Thus Cladocera of the 8 μg dry wt size group at 20° C and food levels of 50 mg dry wt/l spent anaerobically 38 mcal/mg dry wt/h and Eudiaptomus under similar test conditions spent 19 mcal mg dry wt/h which are 49 % and 33 % respectivelly of the energy expenditure.

The responses regarding the energy expenditure, differentiated for each individuals size group under different temperature conditions and excess food concentration, with major implications upon the main functions of the populations as integratory units could be summed up as follows:

— although the assimilated energy reaches its maximum value the assimilability (u^{-1}), decreases as food concentration increases.

— filtration rate decreases inducing conditions of gradually increasing hypoxia.

— oxygen consumption is limited by the decreasing volume of the filtered water and indirectly by food concentration,

— the energy expenditure for maintenance is partially obtained by anaerobic processes.

References were made earlier to a state unlikely to occur in natural ecosystems but which could be induced by a severe deficit of oxygen and food levels below 5 mg dry wt/l.

In the present work an experimental model has been used in order to set out an important mechanism by which filtering Cladocera respond in conditions of excess food and lack of oxygen, characteristic of strongly eutrophic natural ecosystems.

Individuals belonging to species of Cladocera with an average size of 8 μg dry wt have been acclimated to a medium characterized by a food level of 5 mg dry wt/l and an oxygen content that was decreased gradually from 80 mm Hg down to 20 mm Hg. The temperature was maintained at 20°C during the experiment.

Under conditions of 80 mm Hg, the filtration rate was of 19 ml/mg dry wt/h and oxygen consumption reached 29.7 μg O_2/mg dry wt/h.

Below 60 mm Hg, the filtration rate decreased to approximately 4 ml/mg dry wt/h for a period of about 20 hours after which the filtration rate increases again up to a level of 25 ml/mg dry wt/h. The animals reaching this value for filtered volume, higher than initial values, possess haemoglobin. Kring and O'Brian [5] following the effect of reducing oxygen concentration of the filtration rate of *Daphnia pulex* have shown a similar response to oxygen levels below 3 mg O_2/l. These authors have shown that under conditions of persistent and increasing hypoxia, the concentration of haemoglobin increased up to a value of 50 mg/g dry weight. One is naturally going to ask oneself as to the significance of this response and the varying levels of partial oxygen pressure at which this process is triggered. The answer to this question could only come from analysing the effect of fluctuating oxygen concentration upon the flow of energy.

Thus, from our results it can be seen that the oxygen consumption at an oxygen concentration in water of 60 mm Hg is 27.9 μg O_2/mg dry wt/h, which is equivalent to 47 % of the total solved oxygen in the total volume of filtered water (19 ml).

It was shown that lacking efficient oxygen transporting pigments the animals can not take up more than 35 % of the available oxygen from the medium [10], [6].

Thus, in order to maintain the same level of activity the animals would have to waste part of the assimilated energy through anaerobic processes. In the critical situation when the oxygen deficit is accentuating, the increasing of the filtration rate would bring about an increase in the energy required for activity at a rate that would surpass the rate of oxygen uptake from the medium. An answer compatible with individual survival and thus also of the population, would be a temporary decrease of filtration rate with simultaneous synthetis of haemoglobin followed by increasing the filtration rate to a value higher than the initial rate, ensuring more efficiency in supplying the necessary oxygen and consequently a positive value of the energy balance.

Such an interpretation of our results and those of the mentioned workers, made us consider that triggering of the haemoglobin synthesis

occurs when the partial oxygen pressure reaches such a value that would lead to a negative energy balance.

This mechanism of haemoglobin synthesis, found for the species of Cladocera studied by us, could also explain the results obtained under experimental conditions of lack of oxygen and excess of food.

Since data concerning the effects of lack of oxygen and excess food could easily be obtained if the mathematical models from table 1 are applied, only the main mechanisms of response characteristic of this state would be summed up :

— the energy assimilated corresponds to the maximum capacity for food digestion whereas assimilability decreases with increasing food concentration.
— haemoglobin synthesis is synergistically determined both by food concentration levels from reduction of filtration rate and by the effect of oxygen deficit on the energy flow.

It must be mentioned that for our experimental model where food concentration had a value of 40 ± 5 mg dry wt/l and PO_2 had a value of $20\pm\pm3$ mm Hg, haemoglobin amounted to 60 μg/mg dry wt as determined by absorption at 540 nm and by the coefficient of molar extinction of 10,800 M^{-1} cm^{-1} according to Gersonde et al. [2].

Eudiaptomus gracilis did not survive under the conditions on the experimental set-up characterized by marked oxygen deficit and excess food since it lacks the mechanism of haemoglobin synthesis which could ensure a positive energy balance.

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