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LES CRITÈRES DE L'ESPÈCE. LE CRITÈRE MORPHOLOGIQUE

PAR

EUGEN V. NICULESCU

The author shows that all the critics brought to the morphological criterion are not just, and are absolutely unfounded. These critics were made either ignoring the real morphological data of the insects or noticing the phenomenon only in some groups — usually birds and fishes and afterwards trying to generalize the observations at the whole animal kingdom. The author presents numerous proofs of the great value of the genitalia as a very certain and precise morphological criterion in identifying and delimiting the species and genera.

Nous avons montré dans un autre travail [7] que la notion d'espèce morphologique n'est pas correcte puisque, dans la nature, il n'y a pas d'espèces morphologiques. C'est pourquoi nous ne présenterons pas ici le critère d'espèce morphologique, mais le critère morphologique d'espèce.

Ce critère a commencé à être utilisé dès le temps de Linné et continue aujourd'hui d'être dans l'objet de l'attention de tous les zoologistes — même de ceux qui s'en méfient et qui veulent le remplacer par le critère physiologique.

La critique du critère morphologique et son remplacement par le critère physiologique est complètement sans fondement. Elle a été faite sur des positions erronées car on a voulu généraliser les observations faites sur quelques groupes d'animaux — oiseaux — et poissons en général en ne tenant pas compte des insectes. Et quand ont été faites des incursions dans le monde des insectes, les auteurs respectifs ont présenté des critiques non fondées. Leurs conclusions ont été acceptées par un grand nombre de zoologistes et même par certains lépidoptéristes mais sans une analyse sérieuse. Il faut préciser que le problème de l'espèce n'est pas le même dans tout le règne animal. Chaque groupe a ses problèmes spéciaux ; la structure toute spéciale de l'insecte, avec la morphologie si particulière de l'armure génitale pose des problèmes nouveaux parce qu'inexistants chez les Vertébrés. Une juste appréciation du critère de l'espèce ainsi qu'une bonne définition de l'espèce, doit nécessairement tenir compte de l'armure génitale des insectes. Mais ceux qui se sont occupés tout spécialement du problème de l'espèce n'étaient pas des entomologistes mais des biologistes, ichthyologistes et ornithologistes. Ceux-ci, ignorant les eonnées de l'Entomologie, se sont limités, naturellement, aux poissons dt oiseaux où le problème de l'espèce est tout autre qu'en Entomologie. C'est pourquoi nous pensons que le moment est venu de réhabiliter le critère morphologique et de faire la lumière sur ce problème. A cette fin nous allons analyser le critère morphologique en toute objectivité et nous

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examinerons, de même objectivement, toutes les critiques apportées pour voir dans quelle mesure elles sont justes.

D'abord nous tenons à préciser qu'il y a un grand nombre de caractères morphologiques utilisables en taxonomie *, mais tous n'ont pas la même valeur en diagnose [12]. C'est pourquoi quand on critique le critère morphologique doit-on préciser de quel caractère il s'agit. On ne peut pas comparer l'habitus ou les palpes à l'armure génitale. Si, en utilisant exclusivement l'habitus il y a des erreurs systématiques qui se commettent, il n'est pas juste d'exploiter ces erreurs pour discréditer le critère morphologique ; si l'armure génitale avait été examinée et justement interprétée, de telles erreurs ne se seraient pas commises. De même, il n'est pas pertinent d'exploiter les erreurs des lépidoptéristes inexpérimentés qui ne pouvant distinguer un ♂ d'une ♀ ont commis, au passé, des erreurs systématiques. Celles-ci doivent, certainement, être découvertes et corrigées, mais elles ne peuvent pas être utilisées au discrédit du critère morphologique.

Nous allons maintenant énumérer les critiques apportées au critère morphologique et les analyser l'une après l'autre.

1. La première critique concerne les erreurs systématiques commises par les amateurs qui ont décrit comme espèces distinctes les deux sexes d'un papillon. On a dit que souvent entre les mâles et les femelles il y a de si grandes différences morphologiques que le critère morphologique ne peut être utilisé !

2. La même déficience du critère morphologique a été constatée lorsque les deux générations d'une espèce ont été considérées comme des espèces distinctes.

3. La troisième critique se refère aux énormes différences morphologiques entre les premiers états et l'imago ce qui ferait le même critère morphologique inutilisable !

4. Enfin on a observé parfois que les différences dans l'habitus, entre les individus d'une même espèce, sont beaucoup plus grandes que les caractères spécifiques, ce qui diminuerait la valeur du critère morphologique. Ce sont ces critiques qui ont constitué le fondement de l'affirmation suivant laquelle le critère morphologique est vulnérable et partant doit être rejeté et remplacé par le critère physiologique (mixiologique). Est-ce-que les quatre arguments précédents sont valables ? Nous devrons les examiner de plus près pour voir s'ils sont justes ; en deuxième lieu il faudra aussi examiner le critère mixiologique pour nous convaincre de sa valeur et s'il peut remplacer le critère morphologique. Le premier de ces problèmes sera abordé ci-après, le deuxième fera l'objet d'un travail ultérieur.

Discutons maintenant la valabilité des quatre critiques mentionnées ci-dessus.

1. Au premier point il s'agit des différences importantes entre les deux sexes ; parfois ces différences sont si grandes que les deux sexes ont été décrits comme espèces et même comme genres distincts. Mais qu'est-ce-que cela prouve ? Rien sinon le manque d'expérience des lépidoptéristes qui ont fait de telles confusions. Quand nous identifions deux espèces nous

* Nous allons présenter dans un autre travail tous ces caractères morphologiques et nous montrerons, pour chacun en particulier, son importance pour l'identification des espèces.

ne comparons pas le mâle d'une espèce avec la femelle de l'autre espèce, mais le ♂ de l'une avec le ♂ de l'autre (ou les femelles des deux espèces). Or, pour pouvoir faire une telle comparaison, le chercheur doit savoir distinguer un mâle d'une femelle. Si l'habitus ne nous aide à faire une telle distinction, nous devons recourir à l'armure génitale qui résoud immédiatement et sans difficulté le problème. Si le chercheur qui a commis l'erreur n'avait pas de connaissances élémentaires de morphologie et a confondu les deux sexes, est-il juste d'affirmer que le critère morphologique est déficient ?

2. Nous aboutissons à la même conclusion quand nous examinons la deuxième critique. L'exemple classique concernant la description comme espèces distinctes des deux générations nous est offert par *Araschnia levana* L. Vraiment les deux générations (*prorsa* et *levana*) de cette espèce sont si différentes dans l'habitus que n'importe qui serait tenté les considérer comme espèces distinctes. Mais nous savons que l'habitus n'est pas l'unique critère morphologique d'espèce et il n'est pas le meilleur. De même que pour le cas précedent, il ne s'agit pas de la déficience du critère, mais de l'ignorance du chercheur, du défaut de la méthode de recherche. Quand l'armure génitale chez *prorsa* et *levana* a été examinée et s'est révélée d'une identité parfaite et ensuite qu'ont été faites des élévages au laboratoire démontrant que *prorsa* d'été descend de *levana* de printemps, la conclusion juste s'est imposé immédiatement : il s'agit des deux générations de la même espèce, considérées auparavant, par ignorance, comme deux espèces distinctes. En Afrique, dans les régions où l'année a deux saisons, les formes de la saison sèche diffèrent parfois considérablement des formes de la saison pluvieuse. Un bon nombre de formes ont été décrites comme espèces différentes parce que les chercheurs n'avaient pas examiné l'armure génitale.

3. Le troisième argument est tout simplement puéril. On a observé que chez un grand nombre d'invertébrés (insectes, crustacés, mollusques) de l'œuf fécondé ne sort pas un être semblable aux parents mais une larve tout à fait différente. Les adversaires du critère morphologique ont été très étonnés de constater d'énormes différences entre les chenilles et les imago ! ! La conclusion ? Le critère morphologique est déficient ! Nous nous contentons seulement de poser la question : quel sens peut avoir la comparaison de la chenille avec l'imago et la constatation « des différences d'âge » ! ?

4. Enfin les variations individuelles, incriminées de même, nous montrent que le critère morphologique s'étend sur une large gamme et que tous les caractères morphologiques n'ont pas la même valeur; ils doivent être utilisés dans leur hiérarchie et non pas au hasard et arbitrairement. Il est vrai que parfois les variations individuelles dans l'habitus sont beaucoup plus grandes que certains caractères constants spécifiques et c'est pourquoi nombre d'entomologistes ont commis des erreurs systématiques puisqu'ils n'ont pas été capables de faire une discrimination entre les caractères morphologiques. Souvent une aberration présente un habitus tout à fait modifié qui peut masquer le vrai caractère spécifique. Ainsi par exemple certains spécimens de *Melitaea phoebe* Den. et Schiff. ressemblent à s'y méprendre avec *M. cinxia* L. Il y a toutefois dans l'habitus un caractère spécifique constant à savoir la 6e tache rougeâtre

de la bande marginale ; cette tache avance beaucoup vers la base de l'aile antérieure, caractère qui ne manque jamais chez aucun spécimen même le plus aberrant [6]. Et quand l'habitus est déficient l'armure génitale donne immédiatement le « verdict » taxonomique car chez toutes les aberrations quelle qui soit l'amplitude de leur variation, l'armure génitale reste identique à elle-même. Comme dans les cas précédents il ne s'agit pas ici d'une déficience de critère, mais d'une déficience de recherche.

Par conséquent toutes les erreurs systématiques commises dans le passé sont dues d'une part au manque d'expérience de certains chercheurs et d'autre part au manque d'une méthode moderne de recherche. Quand l'étude de l'armure génitale est devenue une méthode courante, de telles erreurs n'ont été plus commises. Mais l'armure génitale non plus ne nous dispense pas d'erreurs — si elle n'est pas judicieusement interprétée par un bon spécialiste. Ainsi, par exemple, chez certaines espèces il y a de petites variations individuelles dans les genitalia [8]. Celles-ci ont été prises, parfois, pour des caractères spécifiques. Quand ces espèces ont été invalidées, on a dit que le critère des genitalia était, lui aussi, déficient avec comme preuve les espèces invalidées ! Ici encore nous constatons que ce n'est pas le critère qui est déficient, mais qu'il s'agit du manque d'expérience de l'auteur. D'habitude les variations individuelles dans les genitalia sont insignifiantes ; mais parfois elles ont une certaine ampleur et nous devons les distinguer des vrais caractères spécifiques qui ne sont jamais masqués par les variations individuelles. Le chercheur compétent et correct examine un grand nombre de spécimens et alors l'amplitude de la variation ressort évidemment. Mais un jeune chercheur impatient de décrire des espèces nouvelles, en examinant un seul spécimen, est souvent tenté de prendre une variation individuelle pour un « bon » caractère spécifique et de décrire une espèce « nouvelle pour la science ». Tous ces faits ne doivent pas se répercuter négativement sur le critère des genitalia. La valeur de ce critère est d'ailleurs attestée par les 120 000 espèces dont les genitalia, connus, sont distincts ! Nous considérons donc que les quatre « arguments » mentionnés ci-dessus sont tout à fait gratuits et nous sommes profondément étonnés qu'ils aient pu être présentés par des zoologistes — d'ailleurs compétents dans leur groupe systématique. Nous sommes convaincus que dorénavant de telles critiques ne seront plus formulées et que le critère morphologique sera remis en ses « droits ».

Mais la discussion sur les critères de l'espèce n'est pas close. Nous sommes disposés à participer à ces discussions et pour commencer nous allons répondre à une critique formulée par Zavadski [15] reproduite d'après Mayr (1947) et Iuzepciuk (1958). Dans son travail, Zavadski écrit : « *Il n'y a pas de caractères spécifiques morphologiques, anatomiques, histologiques ou cytologiques qui permettent le marquage de l'espèce elle-même mais non pas aussi celui des autres groupes (intraspécifiques ou supraspécifiques). Il n'y a aucune sorte de degré de distinction «standard» caractéristique de l'espèce. Un seul et même caractère peut servir de critère d'espèce pour un groupe, et chez d'autres groupes de critère de genre, de famille ou même de sous-espèce* ». A mon avis cette opinion n'est pas valable et nous allons le prouver en prenant toujours comme exemple l'armure génitale. Chaque lépidoptériste sait que l'espèce présente *certains* caractères (spécifiques) et le genre *d'autres* caractères (génériques). Jamais un caractère spécifique d'un groupe ne peut être de

caractère générique ou sous-familial dans un autre groupe. Les sous-espèces et les familles n'entrent pas non plus en ligne de compte puisque dans ces taxa l'armure génitale n'est pas utilisable. Si l'affirmation de Zavadski est, peut-être, valable dans la systématique des oiseaux ou d'autres groupes, en tout cas elle ne l'est pas chez les Lépidoptères.

Dans l'armure génitale de ce groupe d'insectes il y a, chez la plupart des espèces, de nombreux sclérites qui fournissent d'excellents caractères spécifiques et qui ne sont propres qu'à ces espèces. Souvent c'est un seul sclérite (uncus, processus superior, gnathos, penis, fultura inferior, etc.) qui est suffisant pour identifier les espèces avec beaucoup de précision et sans aucune difficulté puisque la forme de ce sclérite est tout à fait particulière et ne caractérise pas que l'espèce respective. Il ne se retrouve plus, avec sa forme caractéristique, chez aucune autre espèce de tout l'ordre, et ne caractérise pas non plus un genre ou un tribu dans un autre groupe. Cela est dû au fait que chez les Lépidoptères l'armure génitale présente une variation interspecifique à l'infini et aucune structure ne se répète jamais. Et le caractère spécifique est clairement distinctif du caractère générique en ayant une autre valeur morphologique, une autre « grandeur » [10], [12]. Ainsi par exemple le processus superior chez le genre *Argynnis* (s. l) diffère nettement d'une espèce à autre. Chez *A. pandora* il est long, mince, muni de dents ténues ; chez *A. paphia* il est court, en massue, muni de dents grossières, tandis que chez *A. sagana* il est très court se terminant en trois pointes vigoureuses, etc. Ces caractères spécifiques du sclérite considéré sont des *détails de structure* concernant sa forme et ses dimensions. Le caractère générique est tout autre à savoir l'existence même de ce sclerite (tous les genres n'ont pas un processus superior) ainsi que son orientation. Chez les *Argynnis* il est dirigé vers l'arrière, mais chez les *Euptoieta* son extrémité distale est recourbée et regarde vers l'avant. Dans aucun autre groupe un processus superior long et grèle comme chez *pandora*, ou en massue comme chez *paphia* ne constitue un caractère de genre ! Dans les groupes où le processsus superior existe il a une toute autre structure et chez les groupes où il n'existe pas, il y a d'autres sclérites qui fournissent de bons caractères génériques.

Voici maintenant quelques exemples de caractères spécifiques « standard ».

1. **Uncus** (et les sclérites voisins). Chez *P. mnemosyne* il est accompagné des subunci ; chez *P. apollo* les subunci manquent [3]. Chez *Pieris napi* à sa base il y a, dorsalement, deux renflements arrondis qui manquent chez *P. rapae* [5]. Chez *A. paphia* il est très recourbé ventralement et étroit, chez *A. pandora* plus court, large, muni de grandes dents ressemblant à une crête de coq [6].

2. **Valva**. Chez *P. mnemosyne* pas de sclérite harpe, chez *P. apollo* celui-ci existe. La valve de *P. rapae* est dépourvue d'apophyse sur le bord externe, celle de *P. brassicae* est munie d'une telle apophyse. Chez *C. hyale* la valve a une excroissance conique sur le bord postérieur qui manque chez *C. erate*. *Neptis rivularis* — la valve est dépourvue de harpe, *N. hylas* — la harpe est présente. Chez *M. phoebe* la valve se termine par trois dents, chez *M. cinxia* par une seule dent.

3. **Penis**. Chez *N. polychloros* il se termine distalement par une pointe courte, chez *N. xanthomelas* la pointe est beaucoup plus longue. Chez

M. didyma l'extrémité proximale est beaucoup recourbée dorsalement, apophysis aedeagi absente, chez *M. cinxia* cette extrémité est faiblement recourbée dorsalement, apophysis aedeagi est présente [6]. Nous avons choisi, au hasard quelques exemples seulement; on pourrait apporter d'autres milliers et milliers d'exemples semblables. Tous ces caractères sont seulement spécifiques, propres aux espèces respectives; nulle part ils n'ont de valeur générique.

Nous avons répondu, aussi par les lignes ci-dessus, aux zoologistes qui affirment qu'il n'y a aucun critère, qui *à lui seul*, puisse permettre l'identification des espèces; on dit qu'il faut toujours considérer un ensemble de caractères [1] [2]. Chez les Lépidoptères il n'est pas besoin d'un ensemble de caractères, car dans l'armure génitale nous trouvons toujours un indice sûr de détermination. D'ailleurs, l'armure génitale elle-même, si compliquée, est un ensemble de caractères. Si nous avions sur notre table de travail quelques milliers d'abdomens de papillons, chacun pourrait être identifié, *sans le reste du corps*, et rapporté à l'espèce à laquelle il appartient *. Nous pouvons identifier l'espèce seulement d'après les genitalia et très souvent même d'après un seul sclérite. Le nombre des exceptions à cette règle — quelques dizaines de cas seulement dans tout l'ordre — est si petit, rapporté au nombre total des espèces, qu'il constitue une minorité infime qui ne peut pas amoindrir en rien notre aphorisme : « *Il y a autant d'espèces différents qu'il existe de structures différentes dans les genitalia* » (E. Niculescu, 1961). Il est vrai, la littérature consigne plus de « quelques dizaines de cas », mais à notre avis dans beaucoup de ces cas il ne s'agit pas de bonnes espèces, mais de formes intraspécifiques. En tout cas elles ne représentent rien à l'égard du nombre considérable de plus de 120 000 espèces dont les genitalia sont différents. Les exemples ci-dessus nous montrent jusqu'à l'évidence que dans l'armure génitale il y a de bons caractères spécifiques et génériques et qu'un certain caractère est propre à une certaine espèce; il ne peut pas servir comme critère d'espèce pour un groupe et comme critère de genre, famille ou sous-espèce pour d'autres groupes comme soutiennent Mayr, Iuzepciuk et Zavadski. En nous basant sur ces particularités remarquables de l'armure génitale, nous avons elaboré l'idée d'un *plan de structure* [11] caractéristique pour chaque genre. C'est précisement ce plan de structure qui confère à l'armure génitale une valeur taxonomique de premier rang à l'échelon générique. Tout genre a un certain plan de structure, bien individualisé, que nous ne retrouvons plus ailleurs; il ne se répète jamais. Cela nous a porté d'élaborer l'aphorisme suivant : « *Il y a autant de genres différents qu'il y a de plans structuraux existant dans les genitalia* » (E. V. Niculescu, 1970). Si les ichthyologistes et les ornithologistes connaissaient l'armure génitale si merveilleuse des Lépidoptères, ils modifieraient radicalement leur conception sur l'espèce ainsi que la définition de celle-ci.

* Aux États-Unis de telles opérations ont lieu de temps à autre dans les circonstances suivantes. Un avion vole à quelques centaines de mètres en traînant un grand filet où pénètrent tous les insectes rencontrès. Les ailes des papillons sont complètement déchirées et souvent le beau papillon est réduit seulement à son abdomen. Tous ces abdomens sont ensuite identifiés dans le laboratoire, le chercheur s'aidant de la grande collection de préparations de genitalia existant à l'Institut.

Le critère des genitalia reçoit chaque jour de nouvelles confirmations. On a accumulé jusqu'à présent un volume immense de données qui montrent la primauté de ce critère à l'égard de tous les autres. Les phrases comme celle-ci : « *L'habitus est si variable que toute identification des espèces sans l'aide de l'armure génitale devient tout à fait illusoire* » — peuvent être lues dans des milliers et des milliers de travaux.

Pour conclure nous affirmons donc que le critère morphologique non seulement n'est pas vulnérable et ne doit être rejeté mais au contraire doit être toujours utilisé comme étant le plus précieux, le plus sûr et le plus précis. En outre il est le plus pratique et le plus commode car il permet une identification rapide à l'échelon spécifique et générique. Mais, et nous insistons de nouveau sur ce fait, le chercheur doit faire une discrimination attentive entre les caractères morphologiques, vu qu'ils n'ont pas la même valeur. Le critère morphologique, et surtout l'armure génitale, judicieusement interprétée, avec objectivité, en tenant compte de la hiérarchie des caractères ainsi que des deux principes (*l'équivalence des caractères et le plan de structure*) et manié par un bon connaisseur du groupe, doué non seulement de bon sens mais aussi de probité scientifique, peut rendre de grands services à la taxonomie.

BIBLIOGRAPHIE

1. Beer Sergio, Sachetti Alfredo, *Problemi di Sistematica Biologica*, Ed. Einaudi, Roma, 1952.
2. Mayr Ernst, *Animal species and evolution*, Cambridge, Massachussets, 1963.
3. Niculescu Eugen V., *Fauna R.P.R., Insecta. Lepidoptera. Fam. Papilionidae*, Ed. Academiei, Bucureşti, 1961, **11**, *5*.
4. — Bull. Soc. Ent. Mulhouse, 1961, 1—13.
5. — *Fauna R.P.R., Insecta, Lepidoptera. Fam. Pieridae*, Ed. Academiei, Bucureşti, 1963, **11**, *6*.
6. — *Fauna R.P.R., Insecta. Lepidoptera. Fam. Nymphalidae*, Ed. Academiei, Bucureşti, 1965, **117**, *7*.
7. — Rev. Verviétoise Hist. Nat., 1966, **23**, 6—8, 42—53.
8. — Rev. Verviétoise Hist. Nat., 1967, **24**, 1—6, 3—11.
9. — Mitt. d'Ent. Ges. Basel, 1968, **18**, 27—31.
10. — St. şi cerc. biol. Seria Zoologie, 1969, **21**, *2*, 137—140.
11. — Linneana Belgica, 1970, *4*, *4*, 81—84.
12. — St. şi cerc. biol. Seria Zoologie, 1970, **22**, *4*, 309—319.
13. — Rev. Verviétoise Hist. Nat., 1971, **28**, 7—9, 1—8.
14. — Rev. Roum. Biol. — Zoologie, 1971, **16**, *5*, 309—314.
15. Zavadski K. M., *Teoria speciei* (traducere N. Botnariuc), Ed. Ştiinţifică, Bucureşti, 1963.

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ARCHAIC SPECIES OF TANAIDACEA FROM THE TANZANIAN WATERS WITH THE DESCRIPTION OF A NEW GENUS, TANZANAPSEUDES

BY

MIHAI BĂCESCU

Some new Tanaidacea from the Tanzanian coral reef are reported; among these, a new genus and a new family: g. *Tanzanapseudes* n.g. of the family *Tanzanapseudidae* n. fam., with circularstarlike body, — unique adaptation among Tanaidacea, to the epibionth life on corals and spongiae — and a new species: *Cyclopoapseudes estafricana* n. sp.
Ecological and zoogeographical remarks on these Monokonophora are given; the last genera are new to the Indian Ocean.

During a two-month exploration of the coral reefs from the Tanzanian waters, I also captured several coralobionth or spongiobionth Tanaidacea. In the present note we are especially dealing with a very peculiar representative of this order, namely the genus *Tanzanapseudes* n.g.

Tanzanapseudes n. gen.

Diagnose. Body extremely flattened dorsoventrally, with integument non-calcified, projected in 31 long, triangular, acute expansions, which completely conceal the rostrum, the telson and even the peraeopods. By the expansions richly provided with setae and spiniform laminae, the animal acquires a discoidal stellate shape resembling the Serolidae Isopoda (Fig. 1, *A*).

Pleon non-segmented, totally fused with telson. Chelae symmetrical but dimorphic, without exopodite. In exchange, peraeopod II presents an exopodite this peraeopod is not changed into a burrowing appendage, but is of a climbing type, having strong claws, like the remaining peraeopods.

Chelae ♂ twice as strong as in ♀. Hypospheniums are absent, and the sexual protuberance is hardly visible in ♂.

The antennule is geniculate, with uni-segmented flagelli starting from a second common basilar segment. Antenna without squama with bi-segmented flagellum. Uropods short with 4-segmented exopodite, and 2-segmented endopodite. Three pairs of normal, biramous uropods (♂ and ♀).

In this new genus, the following two species could be identified:

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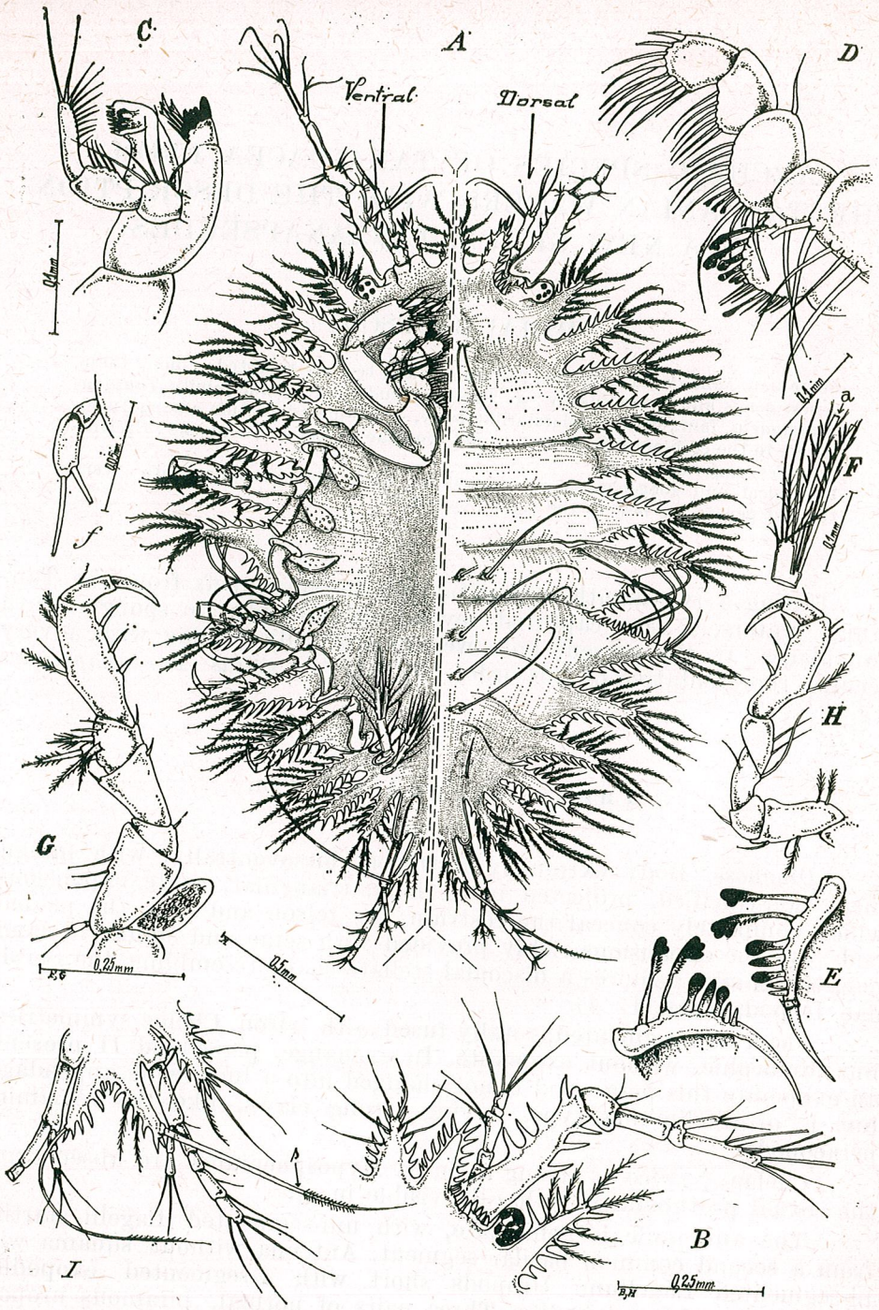

Fig. 1. — *Tanzanapseudes longiseta* n. gen., n. sp. ♀ P. *A*, seen as a whole : right from above, left from below ; *B*, cephalic portion of another ♀ specimen ; *C*, mandible ; *D*, maxilliped I ; *E*, margins of endites, symmetrical, turned up, with phanera opposite to each other ; *F*, tip of antenna figured in *A*, magnified, with the two aestethascae (*a*), sectioned ; *G*, peraeopod II ; *f*, its exopodite ; *H*, peraeopod VI ; *I*, caudal portion of another ♀ ; *s*, agglutinant seta. (In black, chitinous portions).

1. Tanzanapseudes longiseta n.g.n.sp.

(Fig. 1, *A—I*, and fig. 2 *A—G*)

Material. Five specimens : 1 ♂ ad. from *Tubipora* branches, Mbudya, 20.I. 1974 ; 2 juv. fallen from *Acropora corymbosa* soaked in slighthy formalinized water ; 1.XII.1973 — Mbudya ; 1 ♀ fallen from *Cinachyra australiensis* (det. Prof. M. Sara), the spheric, yellow, tangerine size spongia, very frequent in the entire coral area of Tanzania ; 20.XII, 73 ; 1 ♀ juv. from the phreatic sand, Kunduchi, 21.XII. 73.

Description. ♀ pre-adult ; with 4 pairs of small marsupial laminae. Body almost circular, somewhat longer than wide (ratio : 1.06 ; 1.1 ; 1. 22 (♀♀) ; 1.3 (♂). Integument non calcified, translucent, glossy with radial "breaks" just like a broken window glass or like the varnish of an old picture. All around the body the integument continues with 31 radial projections, which lend the new Tanaid a starlike — shape. The distribution of the rays is the following : 3 frontal rays ; (Fig. 1, A), 4 laterocephalic pairs ; 6 lateral pairs ; 3 pleonal pairs and 2 caudal rays. The lateral ones are the longest and partially correspond to the epimeres of peraeionites, with beneath and proximal strong swellings — insertion points of peraeopods.

These rays — unique formations in the Tanaidacea group — ask for a more detailed description, showing as specific characters, a very peculiar armature and phanerotaxia.

In the first place, these rays have all around a variable number — according to site — of pointed non-segmented projections, we'll name spiniform laminae ; a series of pennate, short and especially terminal setae issuing from their base enhance the ability of this crustacean to adhere to the substratum on which it lives. However, the most apart phanera type is represented *by huge setae curved towards* the dorsal and midline of the animal, present solely along some of the rays.

Their distribution is about the following ; on the frontal-lateral rays, 5—6 pairs of spiniform laminae exclusively, continuing the frons by some other shorter ones above the basis of A_1 up to the eyes (Fig. 1, *B*) ; pennate setae are absent ; the midray has 5 pairs of such laminae (one or two distal) and 6 distal pennate setae ; the cephalic rays have about 6 pairs of laminae and pennate setae. The peraeional (lateral) rays with 6—10 pairs of spiniform laminae ; the most numerous are on the epimeres 3 and 4 which aside from the common pennate setae also present 2—3 huge upturned setae, longer than the relative rays and glabrous in their proximal half, whereas in the distal one shey are finely ciliated. Such a seta, but longer, is to be found on each mid-epimere and another two shorter ones, on the midperaeonites, dorsally ; finally, the longest ones are the two setae on the dorsal and of the caudal rays (Fig. 1 *I*, *s*) ; the latter are distinctly upturned anteriorly, reaching the peraeonites. The caudal rays also each with 1—2 pennate setae and about 14 spiniform laminae placed only in their inner portion, the latter also on the bottom of the curvature between them ; their outer edges are glabrous.

Peraeonites about 10 (ten !) times as broad as long (relative adjacent rays, inclusively) are the only distinctly delimited segments. *Pleonites*

from a global whole, without any trace of suture either between them or with the telson; only the 3 pairs of pleopods insertion circles, make us infer that they must have been 3 pleonites.

Telson non delimited by the pleon; the anal aperture is covered and overreached by the bottom of the two caudal rays.

Eyes brown strongly developed, somewhat slightly pedunculate, with over 10 huge, omatidiae (Fig. 1, *B*).

Antennule with first basal segment strong, slightly flattened dorsoventrally, smooth, and bearing 3—4 strong outer spines and 4—5 inner ones (Fig. 1,*B*); the first segment is almost twice as long as the combination of the next two segments. The second segment is fitted in a right angle turning outwards the remaining part of the antenna. Flagelli are uni-segmented, both fixed at the same level on a common segment, the outer flagellum is twice as long and thick as the inner one and has 4 aesthetascae uniseriately ciliated (Fig. 1, *F*, *a*).

Antenna is much shorter and weaker than the proximal, basal segment of the antennule; its basal segment partially overlaps the next segment; scuama is absent. Flagellum, short, bi-segmented. (Fig. 2, *A*). Labrum without projection. Mandible thin, symmetric with a 3-segmented flat, palp, bearing 4 strong teeth at the pars incisiva, with a long pars molaris and a bunch of bifurcate and bifid phanera near the lacinia mobilis. Labium with oval lobes terminating in a strong, clubbed process has two pairs of inner glabrous phanera and long outer cilia. Mandible as in figure 1, *C*: Labium (Fig. 2, *C*) with chitinous expansion.

Maxillule (Fig. 2,*B*) with bisegmented, short palp ending in 2 long terminal seta and two other shorter, non-ciliated, subterminal setae.

Maxilla of the common type.

Maxilliped (Fig. 1, *D*) with a special armature on the endite which bears on its distal edge 2—3 long, clubbed, toothed and chitinous phanera, and also another 4 stick-like or spiniform phanera (Fig.1, *E*), the margins bearing these phanera are slightly turned up like a jacket collar, so that the 2 symmetric ones remind of the normal position of the pharyngeal teeth in fishes (Fig. 1, *E*). On the free, infero-inner margin a single crotchet and 4 simple setae. Caudally and distally, a strong hairy seta which obliquely goes beyond the distal, chitinuous phanera. Epignathus with a distal ciliated cirrhum

Chelipeds (Fig. 2, *D*). The first pair of peraeopods are perfectly symmetrical, equal (Fig. 1, *D*); also flattened dorsoventrally, they do not exceed the stellate outline of the body not even in ♂. Exopodite absent. Basis, ischio-merus and carpus of subequal length, whereas the propodus is distinctly longer. The carpus bears 4—5 long, simple setae (on the inner edge) and only 2 in juveniles; propodus with many setae in the terminal half and a cluster of blunt phanera on the claw margins; the claw of the dactylus is longer than the half of its basis; on the latter, 3 subterminal setae and 3 inner teeths. The margins of the palm and the outer curvature of carpus are strongly ciliated, which in juveniles is not found.

Peraeopod II (Fig. 1, *G*) is somewhat stronger than the others, but just like them it is of the climbing type, with a small exopodite set

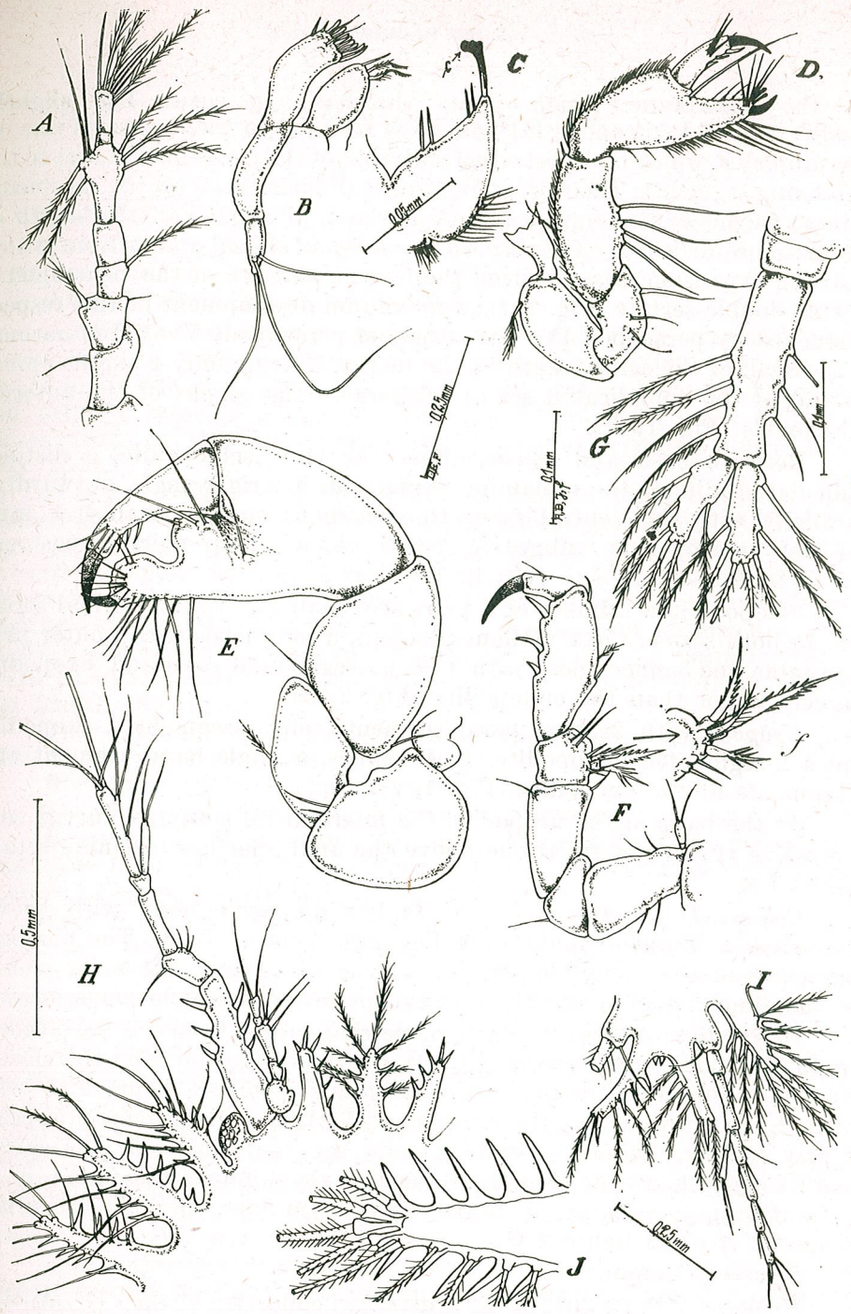

Fig. 2. — *A—G*, *Tanzanapseudes longiseta* n. gen., n. sp. (*A—D* = ♀; *E—G* = ♂) *A*, antenna; *B*, maxillule; *C*, lobe of labium; *c*, chitinous expansion; *D*, peraeopod I (cheliped) ♀; *E*, cheliped from a ♂ nearly equal in size with ♀ represented in figure *D*, by same magnifier; *F*, peraeopod II; *f*, the complicated armature of its carpus; *G*, pleopod III. *H—J*, *Tanzanapseudes langi* n. sp. *H*, cephalic portion, magnified; *I*, caudal extremity; *J*, details in a pleonal ray.

on the membrane which joints the basis to coxa. The slightly smaller size of Peraeopods III and IV is not due to the decreased size of the propodus, which is almost equal with that of the peraeopod II, but to the remaining segments. Their propodus has in all instances 3 (in juv. 2) sternal spines; carpus of peraeopods II—IV is short, of about half the length of merus, more or less rectangular with a series of dorsal spines from which a strong expansion arise, bearing the largest phanera of the peraeopods: 2 setae double-serrate (Fig. 2, *f*); a maximum development in this respect is reached by peraeopod IV. The carpus of peraeopods V—VII is normal, cylindrical, of the same length as the merus, bearing only 2 simple spines instead of the complicated set of phanera in the carpus of the anterior peraeopods (Fig. 1, *H*).

Between basis and merus, above the thin ischiopodite, a distinct geniculation directs the remaining peraeopods in a right angle, outwardly; parallelly with the ventral face. In specimens smaller than 1.4 mm, the last peraeopod is embryonic, nondeveloped. Four pairs of oostegae are to be found, obvious even in 1.3 mm ♀♀.

Pleopods in a number of 3 pairs are small but with a normal structure in both sexes: Coxa without phanera, a long basis with 4 outer pennate setae and 3 inner ones, with two unisegmented rami and exopodite scarcely larger than the endopodite (Fig. 2, *G*).

Uropods with 2 short basal segments, one 4-segmented exopodite and a 2-segmented endopodite. In juveniles, a single basal segment and 3 segments at the exopodite (Fig. 1, *I*).

At the basis of the uropod in the infero-distal corner, a curved seta is crossing the symmetrical one above the anal aperture (cleaning seta?) (Fig. 1,*I*,*s*).

Colour. Tanzanapseudes longiseta has an ivory body with brown tints after a complete removal of the mud from its back. The masticatory parts of the mandible, the top of the labial lobe, the inner end of the maxilleped endite, and the claws of peraeopods are chitinous, brown.

Description ♂ (Fig. 2, *E*,*G*). Somewhat larger than the ♀♀ (according to the single ♂ ad. available), the ♂ differs from the ♀ by the chelae, twice as strong and practically not flattened (compare figure 2, *D* with figure 2, *E*, drawn under the same magnifier), where the female appears slightly smaller. Segments without cilia are more inflated, [the carpus hardly longer than wide (Fig. 2, *E*), lacking the 4 long setae found in ♀. Without hypospheniums, it bears only a minute genital tubercule. Pleopodes ♂ as in figure 2 *G*.

Size = 2—3 mm.

Holotype ♀ P, no 301, in the crustacean collection of the « Gr. Antipa Museum »; paratype ♂, ditto, no 302.

Geographical distribution. Epibionth species (commensal?) in the eastern tropical African waters; for the time being, found only at Kunduchi and in the reefs of the Mbudya Island (Tanzania).

2. Tanzanapseudes langi n.sp.

(Fig. 2, *H*—J)

Material. A single ♀ preadult, on a sponge from Mbudya.

Description (♀). Body circular, hardly longer than wide. Integument non-calcified, ivory, without varnish breaks.

Also shows 31 radial projections.

Among the frontal rays, the midray presents both setae and spines (about 3 pairs), whereas the lateral rays have only spiniform expansions on the inner side (2—3 lateral, 3 apical). The first cephalic ray has spines and pennate setae (4—5 pairs); the second and third ones, only with spines; rostrally, spiniform laminae and setae on the caudal edge (4 pairs) (Fig. 2, *I*).

The first 4 lateral rays have spiniform laminae on the rostral edge exclusively, and laminae + setae on the caudal one; along the last two rays the arrangement is converse (Fig. 2, *H*). The pleonal rays 1 and 2 have rostral spines and setae, whereas caudally, only spines; the third ray with setae and spines on both edges. The 2 caudal rays have pennate setae and spines only on the inner edges. The spiniform laminae are numerically much reduced as against *T.longiseta*: only 4—5 pairs instead of 6—10 as in the latter. The pennate setae are reduced both in number and length (fig. 2 J).

The antennule and the remaining appendages, about similar to those in the previous species, somewhat finer.

Holotype: ♀ "Gr. Antipa Museum", no 303.

Geographical distribution: Tanzania, Mbudya.

Remarks: It differs from the previous species by the lack of the long, close-fitting setae, by the short pennate setae and by the reduced number of spiniform laminae arming the rays.

General remarks. Just like the specimens of *Cycloapseudes* captured in the same samples, the live representatives of the genus *Tanzanapseudes* are hardly distinguished, as they are completely covered with mud and detritus; moreover, when one presumes an animal in his mud clod, a very strong brushing is necessary to separate it from its camouflage. Upon this operation, part of the long dorsal setae of *T. longiseta* are necessarily torn out, these setae playing the same role of fitting the detritus as in *Cumella coralicola* Băc.

In this genus there is no possibility to distinguish a rostrum or a telson in the standard sense of the group, because these body extremities are concealed by the radial projections. The adaptative economy of species exerted a maximum reduction of the long appendages (antennules, uropods) and the peraeopods do not go beyond the animals perimeter.

Tanzanapseudes cannot be referred to the Metapseudidae family, recently created by Lang to which he confered as a characteristic feature, a 2—5 segmented pleon, nor could it be included in the Pagurapseudidae, where species with unaltered type of peraeopod II have been included (whence *Hodometrica* should also be removed).

Such a spectacular feature — *the transformation of the body into an adhesive, fringed star with 31 rays*, under which the long peraeopodal

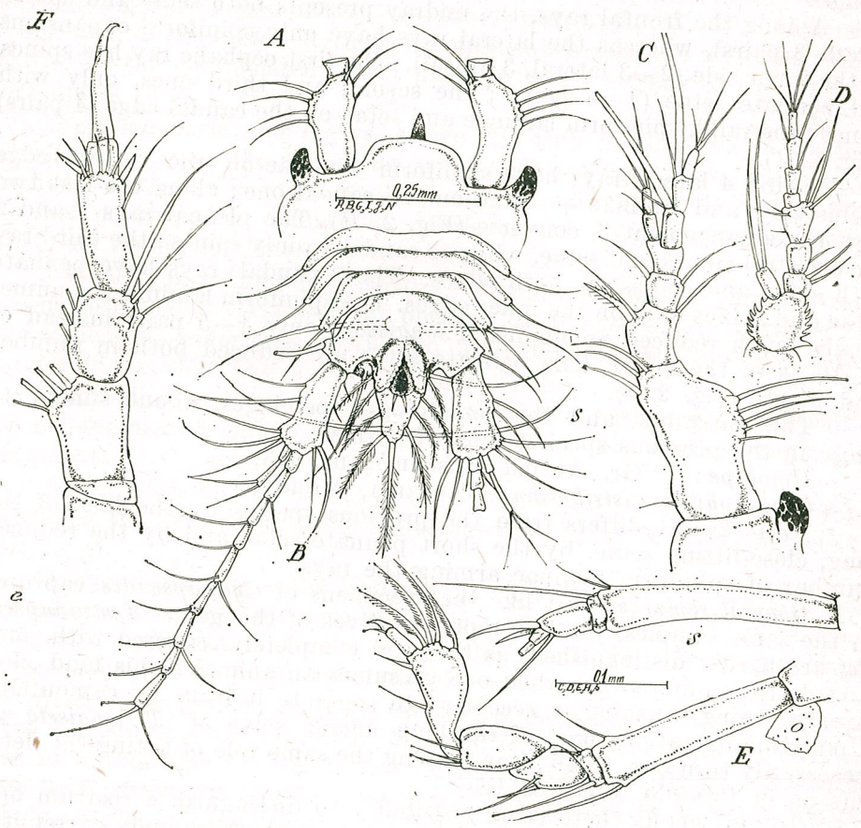

Fig. 3. — *A—F*, *Cyclopoapseudes estafricana* n. sp. ♀ ovigerous. *A*, cephalic extremity; *B*, caudal extremity, seen from beneath; *s*, seta; *C*, antennule; *D*, antenna; *E*, peraeopod V; *s*, ditto, symmetrical with anormal distal portion (*o*, oostegite); *F*, extremity of the last peraeopod.

claws manipulate, just as in parasite isopods, as well as the complete flatness, and the fusion of pleonites are characters unique in the Tanaidacean group, which entitles us to feel that the new genus is also a representative of a new family, *Tanzanapseudidae* n. fam.

To the characterization of this new family it should be added: presence of a rudimentary exopodite at peraeopod 2 exclusively, not at the first one (Chela); peraeopod 2 of climbing type non modified, showing a morphology similar to that of peraeopods III—VII; absence of antennal squama and presence of 3 pairs of normal pleopods.

The representatives of Tanzanapseudidae are certainly archaic types, able to survive in the ancient and relatively constant world of the corals.

Among the coralobionth species frequently captured along with *Tanzanapseudes longiseta* we found 2 species of *Synapseudes* (among which one species allied with *S. carinatus* Makk. from the Red Sea) and *Cyclopoapseudes*.

The acquirement of an umbrella or a stellate disk shape in the Tanaidacean group is representing an interesting phenomenon of convergence with the flattened forms of Isopods (Serolidae, Janiridae etc., of Amphipods (*Palinnectus* f. inst. of which we also found a species in the biocenosis of *Tanzanapseudes*), or discoidal Cyclopidae, and is the result of a long adaptation process to the rocky, epibionth — coralobionth — spongicolous life.

In one *T. longiseta* specimen an inextricable texture of filamentous algae (or bacteria?) with chains finer and longer than the large dorsal setae was found. At first sight they lend a wrinkled appearance to the integument over which they extend.

Cyclopoapseudes estafricana n.sp.

(Fig. 3, *A—F*)

Material: 1 ♀ with embryos and 1 juv, from the scraped stones of dead corals of the Mbudya Island reef — Tanzania, 14. XII. 73.

Description (♀). As to shape and morphology the animal resembles *Cyclopoapseudes indecorus* Menzies. Integument non-calcified, extremely fine. The body shows weak tubercles only dorsally, on the pleonites, whence feathered setae are issuing. Rostrum large, well defined laterally, bearing an infero-anterior bristle (Fig. 3, *A*); its margins circumscribe the cone-shaped eyes, bent towards midline, with distinct facets and black pigment.

The five pleonites distinctly separated (Fig. 3, *B*) with long, hairy epimera. Telson trapezoidal, wide, tapering in a conical, triangular tip, exceeding the anal aperture. The telson cone with a single pennate, apical seta and 4 pairs of lateral ones. Two pairs of huge, agglutinant setae are fixed dorsally, two even apically (Fig. 3, *B*, *s*). Other setae of this type are issuing from the tip of the two pleonite tubercles, and another one seta from the margins of each peraeonite, whilst from their midline, another two setae, unequal in length.

Antennule with squama (Fig. 3, *C*) with a large basal segment strongly toothed on both margins, followed by 7 segments, the last fou finer. Antenna (Fig. 3,*D*) of common type. Chela weak, without exopodite just like peraeopod II ; the last, with 3 + 3 bristles on the propodus edges Peraeopod V — *which probably represents the most certain characteristi feature of the genus* — has a peculiar structure, which indicates an evolu tion of its own. The slender basis and the lack of plumose setae lend a finer appearance than that of the remaining peraeopods (Fig. 3, *E*). In our specimen, the symmetrical peraeopod is reduced to a stump. (fig. 3, *s*) Last peraeopod with pointed extremity (Fig. 3, *F*). Uropods with the basis longer than the telson tip, with 9 — 10-segmented endopodite whereas the exopodite is 2-segmented (Fig. 3, *B*).

Colour. Ivory, black eyes.

Size ♀ with 3 embryos = 2 mm ; Holotype ♀, no 304 in the Crustacean collection of the "Gr. Antipa" Museum.

Coenosis : 2—3 of other Monokonophora species, among which *Synapseudes carinatoides* n. sp. dominates.

Remarks. It is for the first time that a representative of the genus *Cyclopoapseudes* has been reported from the Indian Ocean, namely a specimen closer allied with *C. indecorus* Menzies, from the eastern tropical Pacific than with the species from the New Zeeland waters.

If we also take into account that Makkaveeva has recently reported from the Gulf of Aden, *Imitapseudes veleronis* Menzies, *Synapseudes rudis* Menzies and an *Apseudes* allied with *pernix* Menzies — species described also from the Ecuador waters — these unexpected occurrences show a striking kinship between the Monokonophora fauna of the Pacific coral reefs and that of the the West Indian Ocean. This finding — just like the kinship between the Cumacea from the Caribbean and those of the Red Sea (Băcescu [1]) shows the old age of these animals, as well as their presumably Tethys origin.

The species is also epibionth, but less adapted than the previous species, to the coralobionth life ; body less flattened, but still concealed under mud and detritus, which *C. estafricana* gathers and fixes by means of agglutinant, large setae, specific also to other rocky or coralobionth species, as : some *Cumela* (*C. coralicola*), *C. agglutinanta* Băc. [1], some *Imitapseudes*, some Isopoda, lest we again mention *Tanzanapseudes*. We also give the key of the three known species of the genus *Cyclopoapseudes* :

1 (2) peraeopods I (chela) and II with exopodites ; eyes without facets . . . . . *C. diceneon* Gardiner 1973, SE New Zeeland

2 (1) Prp I and II without exopodites ; eyes with well developed facets . . **3**

3 (4) Rostrum uniform, rounded ; first segment of Antenna nonserrated ; three apical setae at the telson and exopodite of uropod consisting of 3 segments . . . . . *C. indecorus* Menzies 1953. Ecuador

4 (3) Rostrum ± rectangular, with proximal segment of Antenna strongly serrated on both edges ; a single apical seta followed by 4 pairs of lateral setae ; uropodal exopodite consisting of 2 segments . . . . . *C. estafricana* mihi — Tanzania

REFERENCES

1. Băcescu M., *New Cumacea from the littoral waters of Florida* (*Caribbean Sea*). Trav. Mus, "Gr. Antipa" Bucarest, 1971, **11**, 4—20.
2. Gardinar L. F., *New species of the genera Synapseudes and Cyclopoapseudes*. Zool. J. Linn. Soc., 1973, **53**, *1*, 25—58.
3. Lang K., *Aufteilung der Apseudiden in vier Familien nebst Aufstellung von zwei Gattungen und einer Art der neuen Familie Leiopidae*. Ark. zool., Stockholm, 1970, (2), **22**, 16, 515—626
4. Menzies R. J., *The Apseudid chelifera of the Eastern tropical and North temperate Pacific Ocean*. Bull. Mus. Campar. Zool. Harvard Coll., 1953, **107**, 493—496.
5. Makkaveeva E. B., *Kacestvennt sostav kolicestvennoe raspredelenie Tanaidovth rakov v Krasnom More*. Vol. Bentos selfa Krasnogo Moriia, Nauk Dum, Kiew, 1971, 88—108.
6. Miller M. A., *The Isopod Crustacea of the Hawaiian Islands* (*Chelifera and Valvifera*)Occ. Papers Ber. Bishop Mus. Hawaii, 1940, **15**, *26*, 295—321.

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CARPOAPSEUDES BACESCUI n.sp. AND *C. MENZIESI* n.sp. (CRUSTACEA-TANAIDACEA) FROM THE PERU-CHILE TRENCH

BY

MODEST GUȚU

Two species, *Carpoapseudes bacescui* n. sp. and *C. menziesi* n. sp. collected during the XIth expedition of R/V "Anton Bruun" from the Peru-Chile trench, are described.

In 1968, Lang described the genus *Carpoapseudes* [3] with four species: *C. longissimus*, *C. oculicornutus*, *C. serratispinosus* and *C. simplicirostris*. Afterwards only Kudinova-Pasternak recorded the presence of *C. serratispinosus* from the northern waters of Alaska bay [2].

The examination of the tanaidological material collected by the XIth "Anton Bruun" expedition in the Peru-Chile trench — a material offered to our Museum by the head of this expedition, Prof. Dr. Robert Menzies and given for study by Prof. Dr. Mihai Băcescu, for which I am deeply grateful — disclosed the presence of two new species belonging to the genus *Carpoapseudes*, which I dedicated to the above mentioned oceanologists.

Carpoapseudes bacescui n.sp.

Material: Station 157, Pacific Ocean, 12°03′S/78°45′W; 3994—4364 m depth, Oct. 28, 1965, 1 ♂ adult.

Description of male (Figs 1 and 2). *Body* (Fig. 1, *A*) strongly chitinized, about 6 times as long as maximal width, with integument provided with short, fine hairs.

Carapace (Fig. 1, *A*, *B*) somewhat triangular with well developed respiratory chambers. Rostrum very broad at its basis, suddenly tapering; on the median portion presents a groove (Fig. 1, *B*). Eye lobes distinctly individualized, without visual elements, extending anteriorly with strong spines. Epistome with spine (Fig. 1, *B*); among chelipeds, a well developed hyposphaenium.

Peraeonites (Fig. 1, *A*). The second (first one free), the third and the seventh much wider than long, the fourth less so; the fifth and the sixth are the longest. Ventrally, each peraeonite presents a small hyposphaenium, slightly larger on peraeonites II and III.

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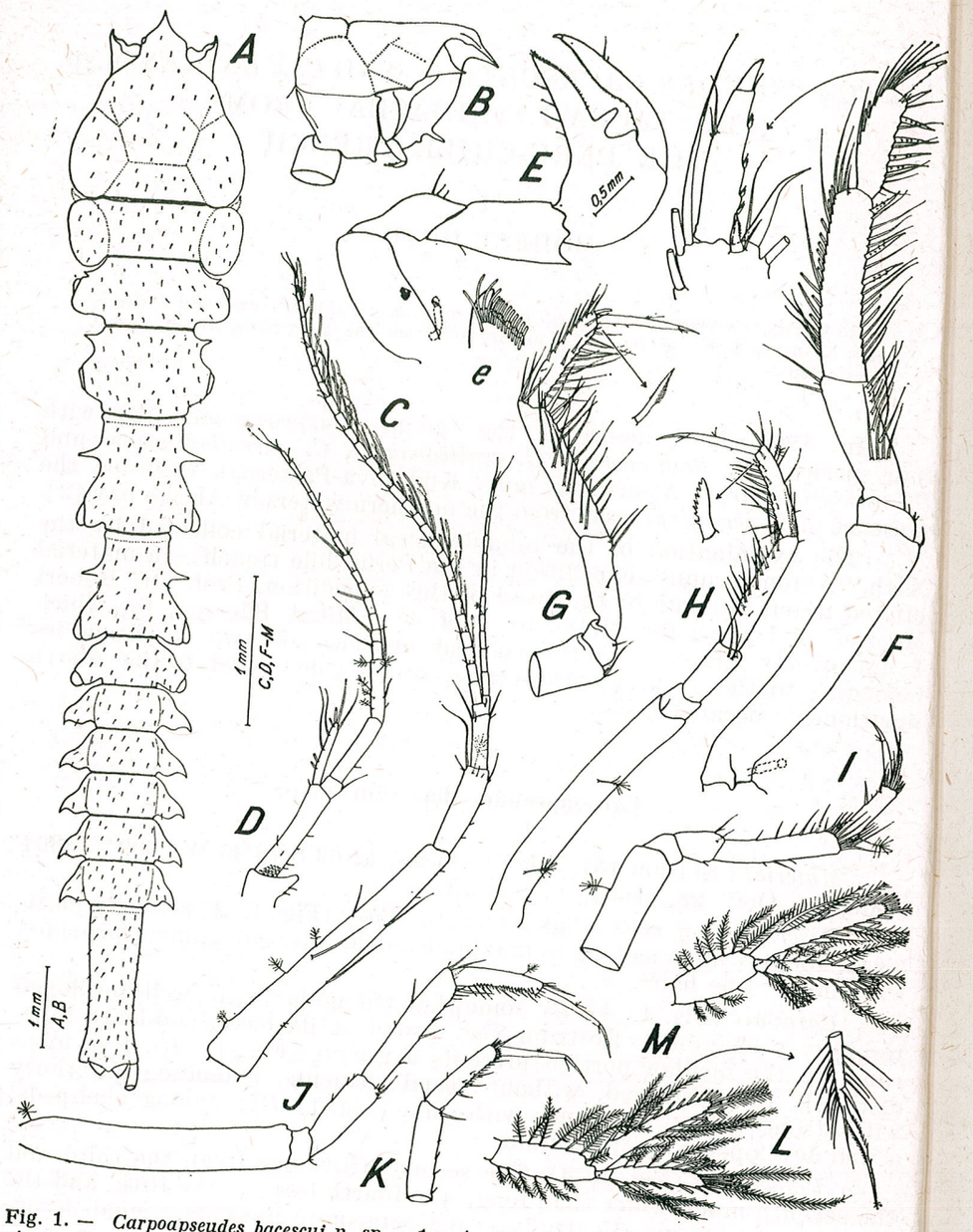

Fig. 1. — *Carpoapseudes bacescui* n. sp. ♂; *A*, general dorsal view; *B*, carapace, lateral view; *C*, antennule; *D*, antenna; *E*, cheliped; *e*, terminal end of propodus with claw; *F*, peraeopod II; *G*, peraeopod III; *H*, peraeopod IV; *I*, peraeopod V; *J*, peraeopod VI; *K*, peraeopod VII; *L*, pleopod I; *M*, pleopod V.

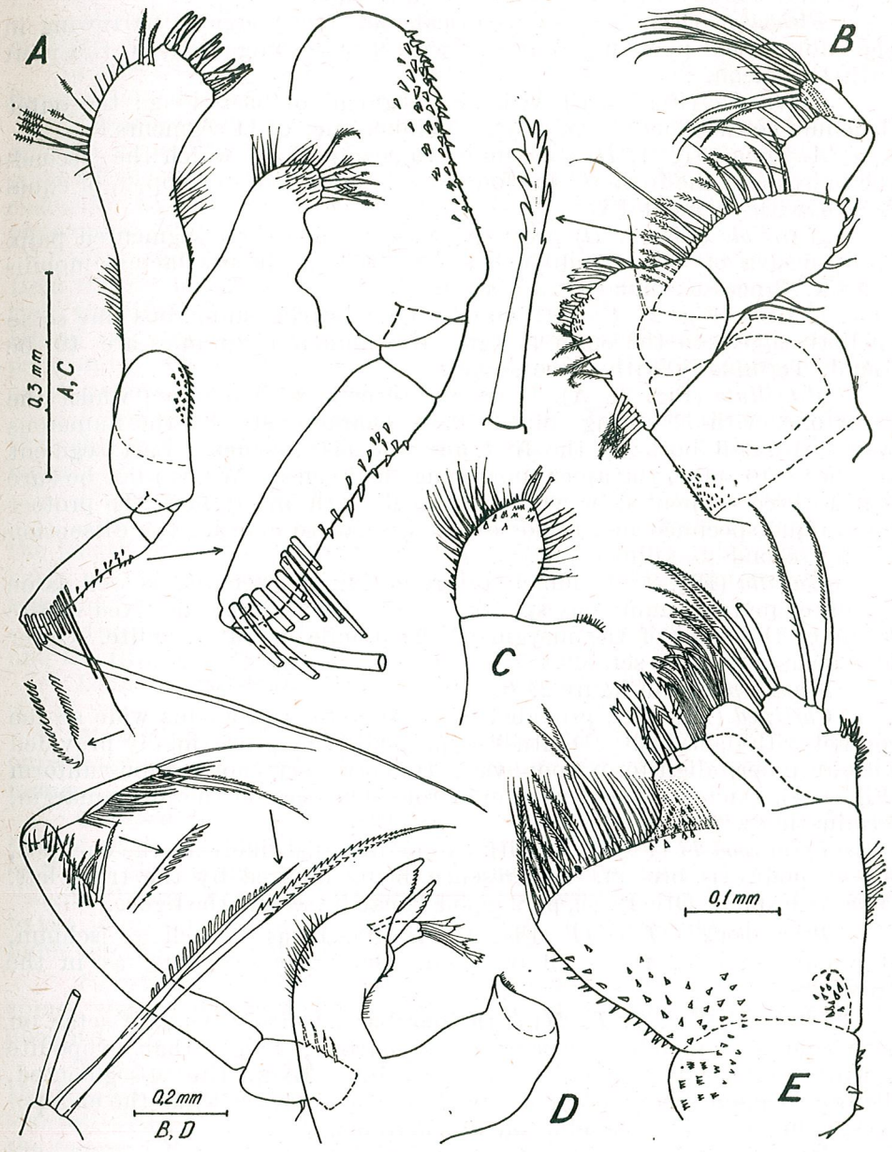

Fig. 2.— *Carpoapseudes bacescui* n.sp. ♂; *A*, maxillule; *B*, maxilliped; *C*, labium, caudal view; *D*, mandible; *E*, maxilla.

Pleonites (Fig. 1, *A*) in a number of five are short and wide. Measured together they have the same length as the combined peraeonites five and six. Ventrally with median small tubercules.

Pleotelson (Fig. 1, *A*) cylindrical and long, slightly narrowing in the subterminal portion. Ventrally with a little bulge next to the joint with the pleon.

Antennule (Fig. 1, *C*) with first segment of basis long; the outer flagellum consists of 28 segments; the inner one, of 11 segments.

Antenna (Fig. 1, *D*) consists of 19 segments of which the second, the fourth and the fifth are the longest. Squama well developed, of equal length with segment five.

Mandible (Fig. 2, *D*) provided with a long, three-segmented palp. Pars incisiva of left mandible has 7 denticles, whereas lacinia mobilis has six. Processus molaris very strong.

Labium (Fig. 2, *C*) with last segment bearing numerous fine setae on both edges, on the outer margin, also numerous spinules are to be found. Terminally with three spines.

Maxillule (Fig. 2, A). Inner endite ends with 5 setae, whilst the outer one, with 11 strong spines. Palp characteristic by the numerous spines situated both on the first and the last segment. Last segment also bears 10 setae, the apical one being the longest. We can not be sure if it is three-segmented or whether it deals with an artefact. To protect the unique specimen in our possession we had to give up the dissection of the second maxillule.

Maxilla (Fig. 2, *E*) lamellate presenting on its basis, as well as on the outer margin, numerous spinules. Rostral margin of the fixed endite is under the level of the margin of the mobile or outer endite. Outer lobe bears numerous setae.

Maxilliped as in figure 2, *B*.

Cheliped (Fig. 1, *E*) provided with exopodite; propodus wide at the contact with dactylus. "Digitus" of propodus long, proximally provided with an inner, digitiform apophysis. Its claw very short, tuberculiform (Fig. 1, *e*). Dactylus long, with numerous denticles on the inner margin, terminating with a small claw.

Peraeopod II (Fig. 1, *F*) with exopodite. Just like with the cheliped, the exopodite is broken, its presence being marked by the trace left. Coxa with spine. Otherwise, peraeopod II can be seen in the figure.

Peraeopods III—VII (Fig. 1, *G*, *H*). Basis as well as ischium, of about equal length in all of them. The other segments, as in the drawings.

Pleopods (Fig. 1, *L*, *M*) with long basis bearing pennate setae on both edges. Endopodite unisegmented, narrow, longer than exopodite bearing long pennate setae on the margins. Exopodite bi-segmented, the first segment being much shorter than the next. Just like the endopodite, it bears numerous setae on the margins.

Uropods broken.

Size: 13 mm length.

Colour: chalk-white.

Holotype ♂, in the collection of Museum of Natural History "Gr. Antipa", Bucharest, no. 277.

Carpoapseudes menziesi n.sp.

Material: Station 95, Pacific Ocean, 8°31′S/81°41′W; 4332—4423 m depth, Oct. 15, 1965, 1 ♂ adult and 1 juvenile.

Description of male (Figs 3 and 4). *Body* (Fig. 3, *A*) about seven times as long as maximal width, covered by fine, rare hairs.

Carapace (Fig. 3, *A*) triangular, with rostrum wide at the basis, scarcely extending anteriorly in a small, acute projection similar to a spine. Eye lobes extending anteriorly, with strong spines, without visual elements. Epistome with a strong spine. Among chelipeds, a long hyposphaenium.

Peraeonites (Fig. 3, *A*) increase in length and decrease in width from the second (first one free) to the fifth. The sixth somewhat shorter than the fifth, the seventh being the shortest. Peraeonites III—VI with lateral spiniform apophysis in the rostral half, increasing in size at the peraeonites which are remote from carapace. Hyposphenium present on all peraeonites, being long enough.

Pleonites (Fig. 3, *A*) also short and wide. Together they are shorter than the combined peraeonite five and six. Ventrally with small tubercules.

Pleotelson (Fig. 3, *A*) long, cylindrical, scarcely shorter than pleon.

Antennule (Fig. 3, *B*). First segment of peduncle measures half the length of outer flagellum and presents an inner tuberosity at the basis, at the level of rostrum (Fig. 3, *A*). Inner flagellum consists of 6 segments, and the outer one of 30 segments.

Antenna (Fig. 3, *C*) has 14 segments of which the second, the fourth and the fifth are the longest. Squama also long.

Labrum, *mandible*, *labium*, *maxillule* and *maxilla* as in figure 4 *A—E*.

Maxilliped with rostral end of palp as in figure 4, *F*, being the only fragment found. Spine of epignathus (Fig. 4, *G*) strong, bearing numerous hairs.

Cheliped (Fig. 3, *D*) with basis comparatively long, and a small sternal spine. Exopodite fitted on a tubercule, consists of three segments, of which the second and the third, particulary long. Propodus with "digitus" long, bearing a digitiform apophysis on the inner side, next to the joint with dactylus. Dactylus long, also presents numerous spinules on the inner side.

Peraeopod II (Fig. 3, *E*). Coxa provided with a moderately large spine. Basis long with an exopodite much smaller than that of the cheliped (Fig. 3, *D*).

Peraeopods IV—VII (Fig. 3, *F—I*) with basis of equal length, the thinner being that of peraeopod VII (Fig. 3, *I*). Peraepod V has the shortest merus (Fig. 3, *G*). Carpus, propodus and dactylus can be seen in the figures. Peraeopod III lacking, being broken.

Pleopods (Fig. 3 *J*, *K*) are in a number of five pairs. Basis well developed bearing 4—5 setae on the inner edge and 2—3 on the outer one. Endopodite unisegmented just like the exopodite, but longer than the latter, both rami presenting long pennate setae.

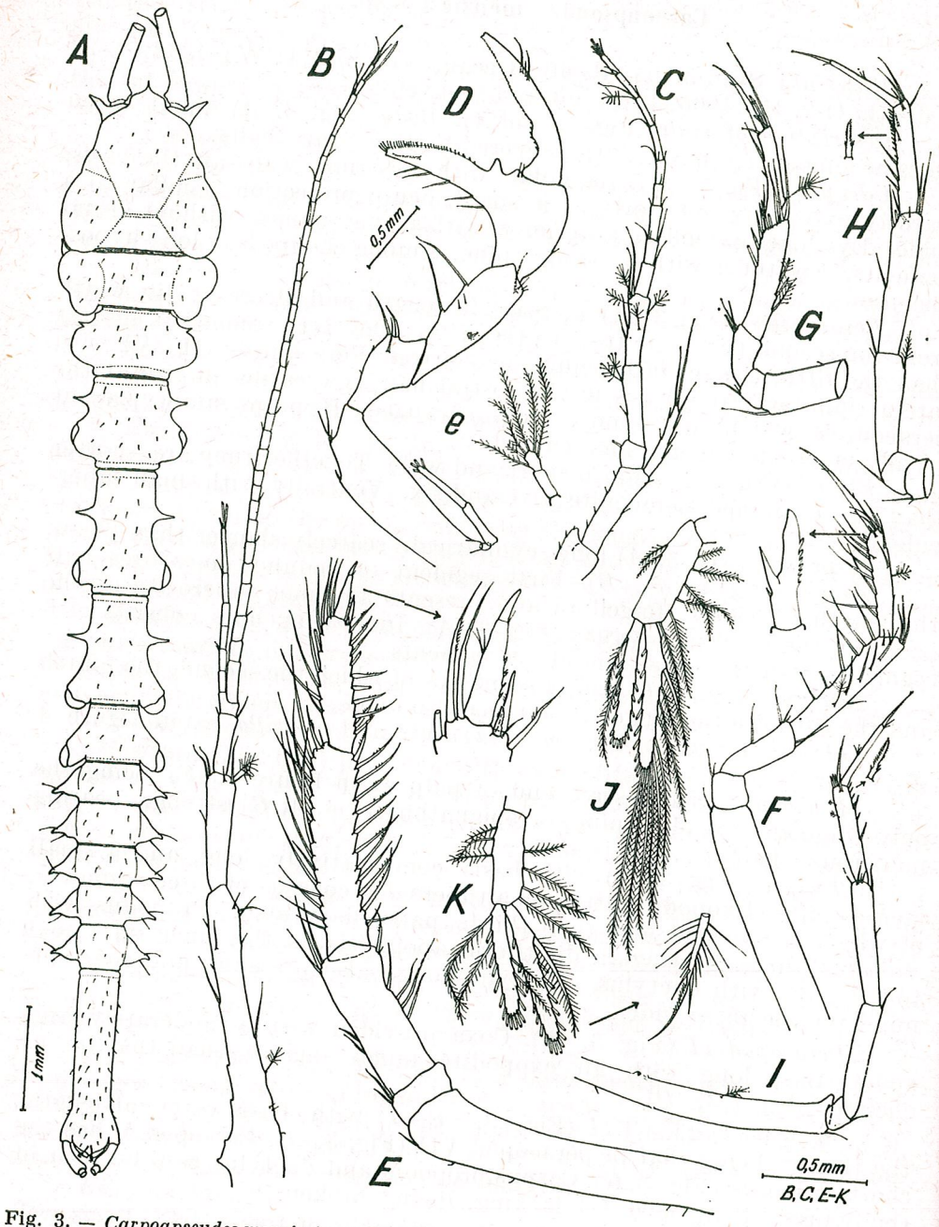

Fig. 3. — *Carpoapseudes menziesi* n.sp. ♂; *A*, general dorsal view; *B*, antennule; *C*, antenna; *D*, cheliped; *E*, peraeopod II; *e*, exopodite of peraeopod II; *F*, peraeopod IV; *G*, peraeopod V; *H*, peraeopod VI; *I*, peraeopod VII; *J*, pleopod I; *K*, pleopod V.

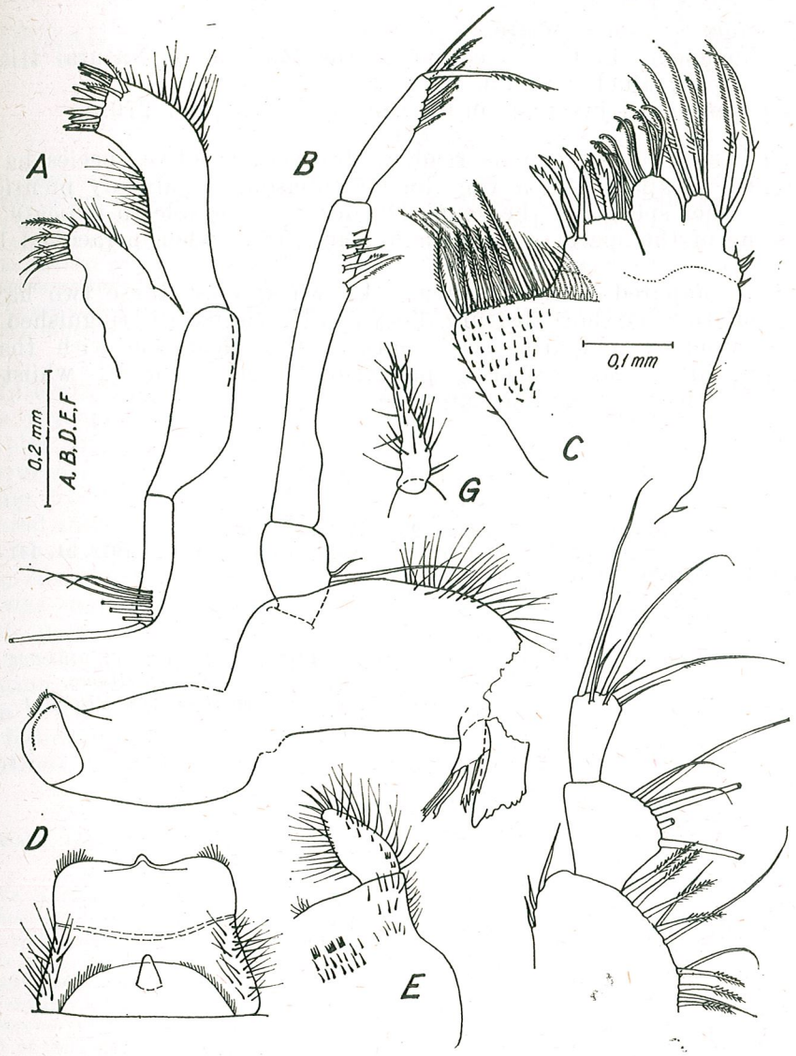

Fig. 4. — *Carpoapseudes menziesi* n. sp. ♂; *A*, maxillule; *B*, mandible; *C*, maxilla; *D*, labrum; *E*, labium (caudal view); *F*, terminal end of maxilliped; *G*, spin of epignathus.

Uropods broken.
Size : 10.4 mm.
Colour : ivory—white.
Holotype ♂, in the collection of the Museum of Natural History "Gr. Antipa", Bucharest, no. 278.
Paratypes : 1 juvenile, in the same collection, no. 279.

Remarks : As it can be readily observed, the two species have a triangular carapace and a very long pleotelson, as already mentioned for the other species of the genus [1], features capable to indicate the belonging of the species to a certain genus, even when peraeopod II is absent.

As compared with the already known species, these two have a characteristic very short rostrum. They can be readily distringuished one from another by comparing the length of the pleotelson with that of carapace, in *C. menziesi* the pleotelson being shorter, whilst in *C. bacescui* longer than the carapace.

REFERENCES

1. Guțu M., Rev. Roum. Biol. — Zoologie, 1972, **17**, 5, 297—305.
2. Kudinova-Pasternak P. K., Trudî Inst. Okeanolog. im P. P. Şirşova, 1973, **91**, 141—168.
3. Lang K., Galathea Report, 1968, **9**, 60—87.

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CEREBRAL GANGLIA REGENERATION IN *LUMBRICUS TERRESTRIS* L.

BY
MARIA CĂDARIU

The nervous tissue of *Lumbricus terrestris L.* regenerates at the expense of the replacement cells of neural ectodermic origin. These cells migrate to the top of the regenerating cone and are activated, acquiring the appearance and the properties of regenerating cells. They divide amitotically and mitotically, remaking the normal cell supply in ganglia, and differentiate afterwards into neurons, neurosecretory cells and glial cells.
The coelomocytes, migrated around the nervous system in the process of regeneration, differentiate into elements characteristic to neurilemma.

Literature data [2—8] [11] [12] show that the origin of nervous system regenerating cells is still questionable ; that their evolution stages in the elements characteristic to the nervous system are not yet specified ; that structural and histochemical aspect of regenerating cells and elements resulting from them was not studied ; that the question of the origin of injured intraneural and extraneural blood system and of neurilemma, covering the nervous system, was not approached. The present paper tries to clear up these questions, studying the regeneration process of cerebral ganglia with n short periods of time, followi ng total and partial extirpations of these ganglia.

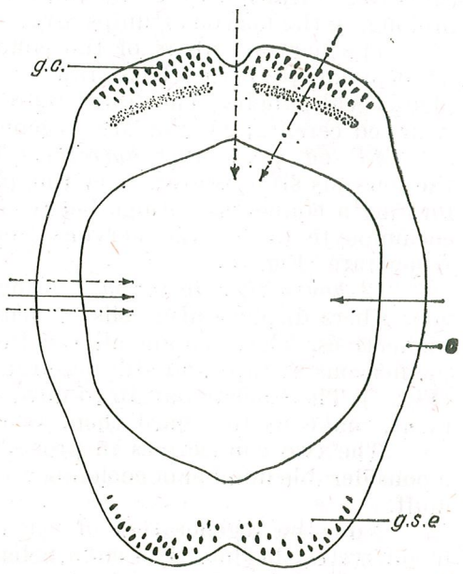


Fig. 1. — Sketch of peripharyngeal ring in *Lumbricus terrestris L.* : *g.c.*, cerebral ganglia ; *g.s.e.*, hypopharyngeal ganglia ; *c*, ring connectives. The arrows show the incisions in order to remove : ⟶ both ganglia ; ⟶ only one ganglion ; ⟶ one cerebral ganglion + half of its pair.

MATERIAL AND METHODS

We worked with adult individuals of *Lumbricus terrestris L.*, during the month of May. Both cerebral ganglia from 300 individuals were removed ; in 50 individuals one ganglion was removed, and in 10 others one was entirely removed as well as the half of the second ganglion (Fig. 1). The anterior part of the animal comprising the first 9 segments was studied within various periods of time following the operation : 3,6, 12, 30, 36, 48, 60, 72, 84, 100, 124, 150 hours, 8, 9, 10, 11, 12, 14, 16, 18, 20, 21, 25, 30, 35, 40, 45, 50, 55 and 62 days. The histologic, cytologic and histochemical examination was carried out using the following methods : fixation in Bouin fluid,

staining with azan, ferric hematoxylin, paraldehyde-fuchsin; fixation in Benoit fluid, staining with acid fuchsin according to Altmann (mitochondria); Kolatchev-Nassonov method (Golgi complex), fixation in Carnoy fluid, staining with methylpyronine green (nucleic acids).

RESULTS

After *removing cerebral ganglia*, the connectives of peripharyngeal ring remain free like two stumps.

Six hours after operation. Cells begin to migrate towards the end of the connectives (Fig. 2). Some small cells, with ovoid and chromatic nucleus, resulting from the nervous tissue of connectives, migrate among the nervous fibers of their cut extremities. Others are large and rounded coelomocytes which change their shape as they get near to the nervous stumps : they become ovoid then prolonged and thinner and they dispose one upon the other, covering the injured surface.

Between *21—30 hours after operation* the coelomocytes migration continues towards the cut end of the connective, covering it like a hood (Fig. 3). The stages of the transformation of normal coelomocytes into fibroid-like cells were described by Zimmermann [12].

36 hours after operation. By addition of coelomocytes, which continue to get transforming into fibroid cells, the hoods become like cords prolonging the nervous stumps over pharynx.

The nervous fibers of the connectives develop through the inside of coelomocytes cords. These fibers are the processes of cells of the hypopharyngeal ganglia. The fibers representing the processes of cells of the removed cerebral ganglia are degenerating.

48—60 hours after operation. The coelomocytes cords prolonging the nervous stumps grow over the pharynx, get nearer and at last fuse forming a connection bridge between them. Through the inside of this coelomocyte bridge the nervous fibers of the connectives continue to regenerate (Fig. 5).

72 hours after operation. During their continuous growth, the nervous fibers displace also cells of neural origin from the cut ends of the connectives. They remain all the time in the regenerative cone top of the nervous stumps and still maintain their initial morphological aspect (Fig. 4). Their behaviour up to now, as well as their subsequent development, make us to regard them as replacement cells.

The two connectives in process of regeneration are surrounded by a considerable number of coelomocytes forming arround them a continuous muff.

Now the regeneration of the injured intraneural and extraneural blood system begins. The endothelial cells at the level of the injury are activated : the nucleus volume increases, the nucleolus is hypertrophied and the cytoplasm becomes basophylic, pyroninophilic.

84 hours after operation. The growing nervous connectives get near to the dorsal median line, over the pharynx.

The process of coelomocyte addition stops. The coelomocytes begin to differentiate into other cellular types : fibroid elements with several nuclei, coming after one another like beads, and binding the ends of the two connectives; myoblasts and connective cells. Many pyknotic nuclei

appear in the coelomocyte muff, which means that degenerative phenomena take place.

100 hours after operation. The ends of the two nervous stumps have got near one to another but have not yet fused. In this moment, the replacement cells in the regenerative cone top of the connective become active. The first sign is the growth of nuclear volume and the nucleolus hypertrophy, followed by the increase of cytoplasm amount which becomes strongly basophilic (Fig. 7). The cell outline remains still rather indefinite. In this way the replacement cells acquire the morphological characteristics of the regenerative cells of cerebral ganglia. In the regenerative cone of the connective we identified 16 regenerating cells.

124 hours after operation. The regeneration process of intraneural and extraneural blood vessels are intensified. The activated endothelial cells get multiplying mitotically.

150 hours after operation. The ends of the two connectives fuse (Fig. 6). The number of regenerating cells has increased. In the regenerative cone of the connective there are 146 regenerating cells. The great number of cells is due to the intensification of the replacement cells activating process, on the one hand, and to the multiplication of regenerating cells themselves, on the other hand. We observed only one mitosis but there are several amitoses (Fig. 8).

8—9 days after operation. Following the more and more intense multiplication, amitotic and mitotic, the number of regenerating cells increases. Now, they are disposed in two layers, forming a beginning of cortex around the neuropil composed of nervous fibers. The ovoid shape of the regenerating cells, having a diameter of 11 μ/2,4 μ, is more evident. Their basophilic cytoplasm contains numerous pyroninophilic granules, few granular mitochondria around the nucleus and massive dictyosomes situated at one of the cell poles (Fig. 9 I).

10 days after operation. The differentiation of some regenerating cells begins. Their ovoid nucleus increases in volume, becomes spherical, vesicular, very poor in chromatin, with 1—2 nucleoli; the amount of cytoplasm — still intensely pyroninophilic — gradually increases, and the Golgi complex remains at a cell pole (Fig. 9 II). Thus, the first neuroblasts appeared (Fig. 10). In the young ganglia cortex regenerated capillaries are observed.

14—16 days after operation. The outline of the new ganglia, more thickened than the connectives of the circumpharyngeal ring, is already defined. Their cortex is rather thick, with several layers of regenerating cells (Fig. 11). Numerous pictures of bipartition of the regenerating cell nucleolus appear. The evolution process of the neuroblasts continues; the cytoplasm amount increases more and more; the Golgi complex develops, maintaining the same location, the nucleolus gets nearer and joins the nuclear membrane, achieving a substance exchange with the surrounding cytoplasm.

The intraneural blood system is already connected to the extraneural system.

18—20 days after operation. Some neuroblasts are differentiated into unipolar neurons. Their abundant cytoplasm contains numerous

granular and filamentous mitochondria and a well developed Golgi complex which extends around the nucleus (Fig. 9 III).

The coelomocyte muff is still very thick. In this deep part it begin the differentiation of the two layers of the tunic which will wrap th regenerated ganglia. The cytoplasm of fibroid coelomocytes in the clos neighbourhood of the nervous system are filled with a substance which ma be stained violet with paraldehyde-fuchsin; then, the entire cell gets th aspect of a violaceous lamella to which the nucleus remains laterally attached; finally the nucleus degenerates. Subsequently, the lamellae joi one another composing the elastic internal layer of the tunica around th regenerated ganglia.

The coelomocytes near to the elastic layer get disposing one afte the other, their cytoplasm loading with substance stainable green wit picroindigocarmine, then the nuclei degenerate. These cells join the elasti layer and constitute the external layer of the tunica covering the nervou system. The forming of the two layers of the tunica covering the regener ated ganglia is a longstanding process which can be followed up to 6 days postoperatively. At the same time the coelomocyte muff become thinner.

21 days after operation. The first neurosecretory cells appear, being few in number and having little neurosecretory material.

25 days after operation. The number of neurosecretory cells has increased, as well as the neurosecretion amount of their cytoplasm. The neurosecretory material release through their axons and in this way the neurosecretion area appears.

The glial cells differentiation begins. Some regenerating cells are attaching to the neuron perikaryon and differentiate into glial cells of type II (Fig. 12) [12].

Between *30—62 days after operation* the nervous system is more and more delimited from the surrounding neurilemma. A very narrow layer results from the coelomocyte differentiation. A great part of coelomocytes has degenerated.

The cortex of ganglia gets thicker and thicker by the increase of the number of neurons and neurosecretory cells. The neurons have differentiated according to areas. The neurosecretory cells differentiate into small cells (a) (Fig. 13), strongly filled with neurosecretion, and large cells with little neurosecretory material. For the time being the small cells are still dispersed. The neurosecretion release through the axons is intensified and consequently the accumulation area becomes more and more evident. The glial cells differentiation goes on. The regenerating cells, in small number, having mitotical properties (3 mitoses in 62 days) are disposed in a layer at the cortex periphery.

In *animals with only one removed ganglion*, the regenerating cells appear both at the end of the cut connective and at the free extremity of the remaining ganglion. They have the same morphological aspect and behaviour as the cells which have entirely regenerated the cerebral ganglia, completely remaking the removed ganglion.

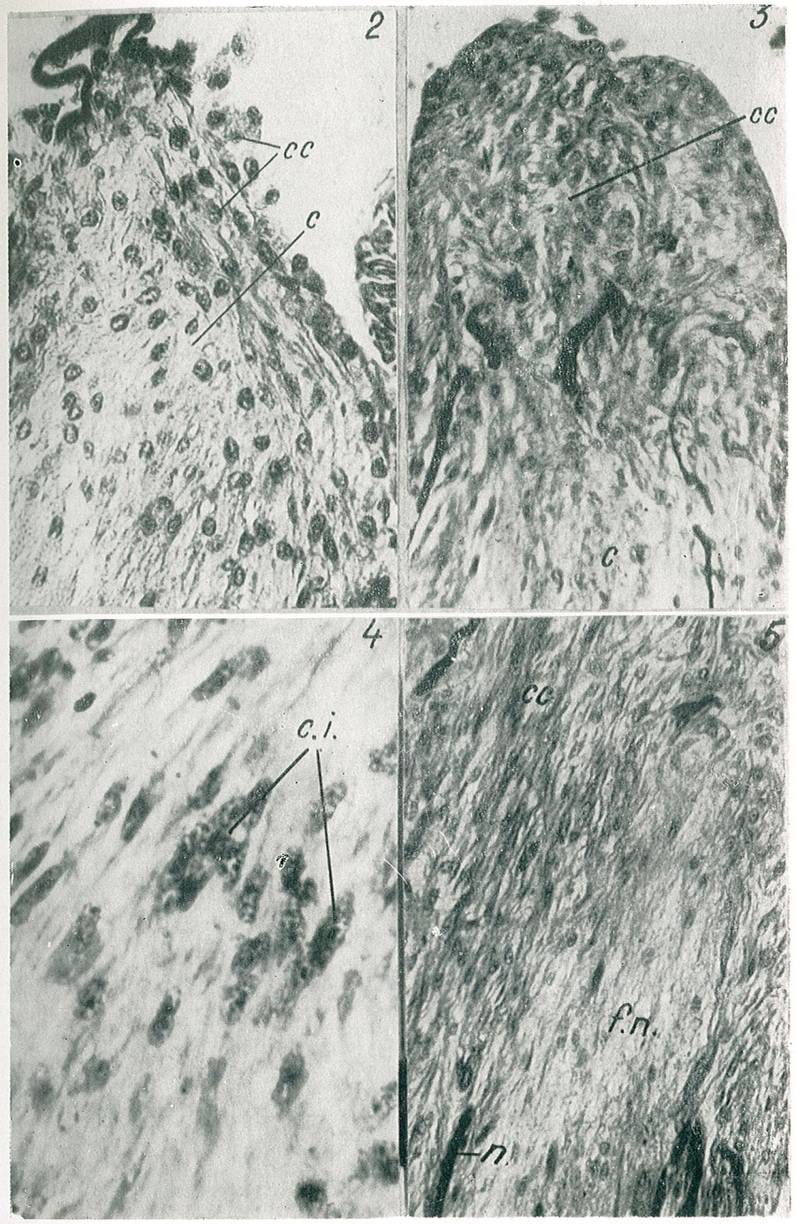


Fig. 2. — The connective end, six hours after cerebral ganglia removing. *c*, connective; *cc*, coelomocytes. Azan method, ob. × 40.
Fig. 3. — Coelomocyte hood (*cc*) at the connective end (*c*), 21 hours after cerebral ganglia removing. Azan method, ob. ×25.
Fig. 4. — Replacement cells (*c.i.*) advance, together with the growth of nervous fibers in the connective. 72 hours after operation. Method of ferric hematoxyline. Ob. ×40.
Fig. 5. — The nervous fibers have grown through the inside of fibroid coelomocyte muff (*cc*); *n*, neurilemma. 60 hours after operation. Azan method. Ob. ×40

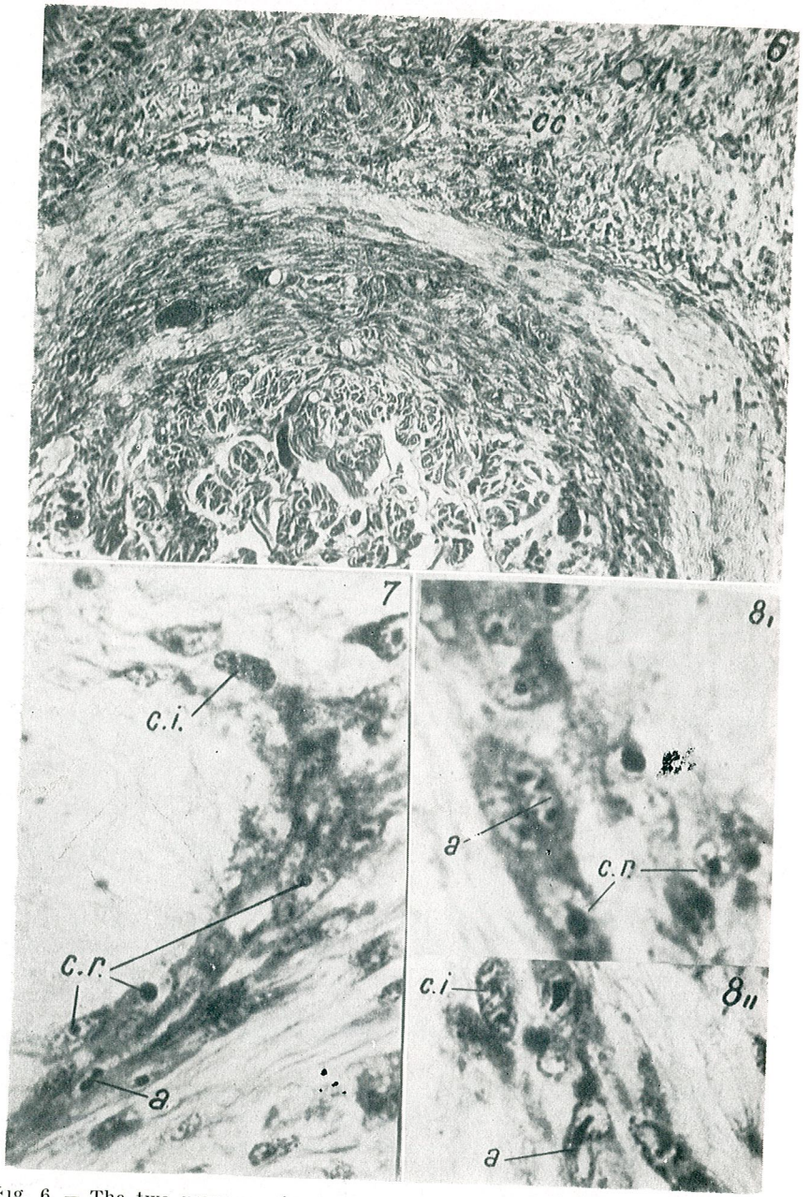


Fig. 6. — The two nervous stumps have fused. 150 hours after operation; *cc*, coelomocyte muff. Method: ferric hematoxyline. Ob. ×10.

Fig. 7. — Regenerative cone at the connective end. 100 hours after operation; *c.i.* replacement cells; *c.r.*, regenerating cells; *a*, amitosis. Method: ferric hematoxyline. Ob. ×90.

Fig. 8. — Regenerating cell amitosis (*a*). *c.i.*, replacement cell. 150 hours after operation. Method: ferric hematoxyline. Ob. ×90.

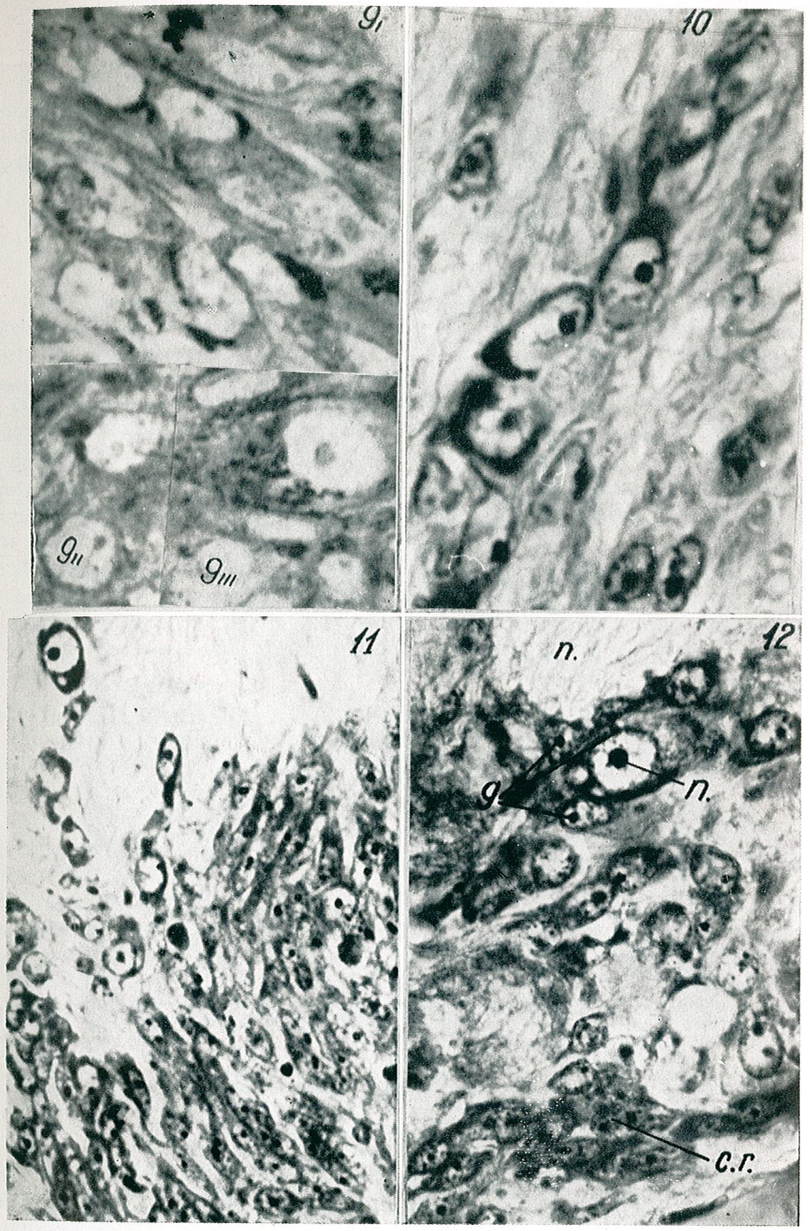
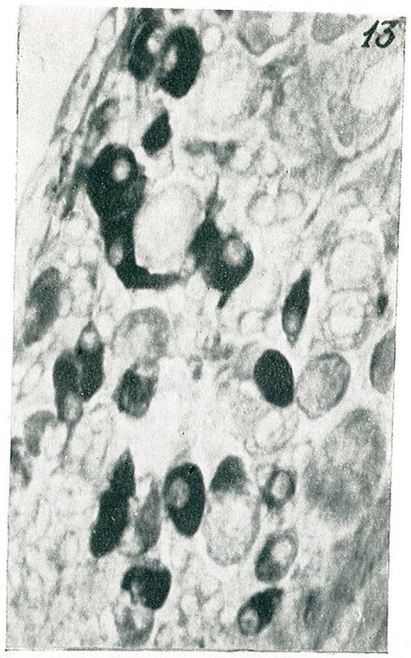


Fig. 9. — Golgi complex in regenerating cells (9_I), in neuroblasts (9_{II})and in the neuron (9_{III}), Kolatchev-Nassonov method. Ob. ×90.

Fig. 10. — Neuroblasts. 10 days after operation. Method: ferric hematoxyline. Ob. ×90.

Fig. 11. — Regenerating cells and neuroblasts in the new ganglia cortex. 16 days after operation. Method: ferric hematoxyline. Ob. ×40.

Fig. 12. — Glial cells (*g*) appear around the neurons (*n*). 25 days after operation. *c.r.*, regenerating cells. Method: ferric hematoxyline. Ob. ×90.



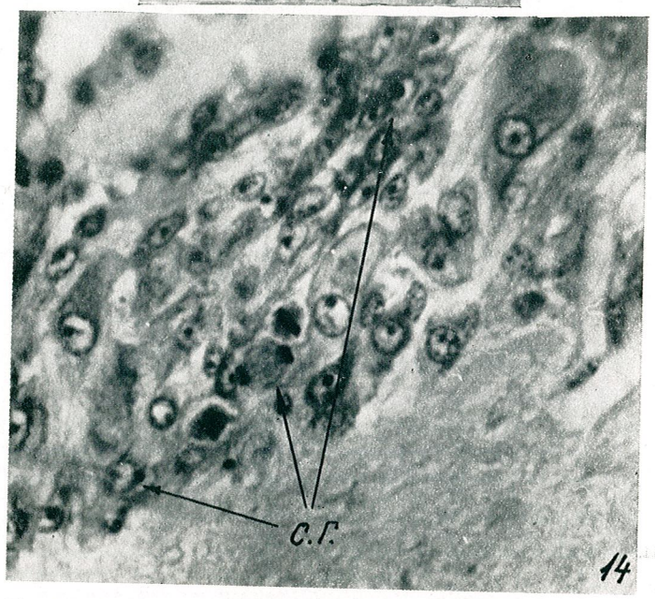


Fig. 13. — Small neurosecretory cells in regenerated ganglia. 35. days after operation. Method : fuchsin-paraldehyde. Ob. ×40.
Fig. 14. — Active regenerating cells and cell in mitosis (*c.r.*) in the cortex of the half ganglion which remained in the same place. 3 days after operation. Method : ferric hematoxyline. Ob. ×40.

By *entirely removing one ganglion and a half of the second one*, the regenerating cells appears at the end of the cut connective and in the cortex of the remaining half ganglion. Figure 14 shows active regenerating cells dividing mitotically among the cortex cells.

DISCUSSION AND CONCLUSIONS

Our researches demonstrate that the nervous tissue of *Lumbricus terrestris* L. regenerates at the expense of some cells of ectodermic neural origin, as Friedländer, Homann, Herlant-Meewis and Zimmermann supposed, in opposition to Hübner's, Nuzum and Rand's and, Schwartz and Hubl's statements. These cells, called "replacement cells" by Herlant-Meewis, remained in a resting state among neurons and neurosecretory and glial cells of the ganglion cortex and among the nervous fibers of the connectives. The replacement cells migrate towards the injured place and they may be recognized only according to their position in the regenerative cone.

The replacement cell activation takes place sooner or later, depending on the seriousness of the injury. It seems that it is determined by the action of some neuro-hormonal factors [4]. These cells replace also the neurosecretory cells, destroyed by holocrine secretion [3].

The cytoplasm pyroninophilia of regenerating cells can be explained by the increase of the ribosome number, as in the neoblasts regenerating the nervous system of *Planaria* [10]. The nucleolus hypertrophy and the increase of the ribosome number demonstrates that structural protein syntheses take place at the level of regenerating cells.

Immediately after their activation, the regenerating cells enter on active multiplication period. This multiplication is achieved not only mitotically, as other authors demonstrated, but also amitotically. It seems that during the first days amitosis prevails, which provides for a more rapid growth of regenerating cells supply. From the 10th day on after operation the differentiation period of regenerating cells in the elements characteristic to the cerebral ganglia begins. They differenciate into : neurons (the first neurons appear 18 days after operation), neurosecretory cells (21 days after operation) and glial cells (25 days after operation).

The injured intraneural and extraneural blood system regenerates at the expense of blood vessels which have remained in the same place, by the mitotical multiplication of endothelial cells. The differentiation of the regenerating cells of the nervous system is conditioned by the vascular system regeneration.

Zimmermann [12] has described the stages of transformation of the normal coelomocytes into fibroid cells and their migration around the regenerating nervous system, but without following their future fate. We found that : the coelomocytes form a thick, protecting muff around the regenerating nervous system ; most coelomocytes differentiate into elements characteristic to neurilemma ; through the inside of this muff the extraneural blood vessels regenerate ; a part of coelomocytes

degenerate. We suppose that certain coelomocytes have a trophic rol feeding the regenerating elements, others have a fagocyting role, destroyi the injured tissues.

REFERENCES

1. Aros B., Vigh B., Acta Biol. Hung., 1961, **12**, 87–98.
2. Aros B., Vigh B., Acta Biol. Hung., 1962, **13**, 177–192; 323–337.
3. Cădariu, M. Studia Univ. Babeş-Bolyai, 1966, **1**, 87–97.
4. Cădariu M., Studia Univ. Babeş-Bolyai, 1972, **1**, 91–98.
5. Herlant-Meewis H., Ann. Soc. Roy. Zool. Belgique, 1956–57, **87**, 151–183.
6. Herlant-Meewis H., Mem. Soc. Endocrin., 1962, 267–274.
7. Hubl H., Archiv. Entwicklungsm., 1952, **145**, 508–572.
8. Hubl H., Archiv. Entwicklungsm., 1956, **149**, 73–87.
9. Pedersen K. J., Z. f. Zellforsch., 1959, **50**, 799–817.
10. Sauzin M. J., Bull. Soc. Zool. France, 1967, **92**, *2*, 313–318.
11. Stephenson J., *The Oligochaeta*, Oxford University Press, 1930, Oxford.
12. Zimmermann P., Z. f. Zellforsch., 1967, **81**, *2*, 190–220.

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PIGMENT-BEARING MULTINUCLEATED CELLS IN THE LIVER OF MALARIAN RODENTS DURING RECOVERY

BY

PAULA PRUNESCU and C. PRUNESCU

In the liver of malarian mice and hamsters occur, during recovery, multinucleated giant cells, overcharged with malarian pigment. They display peripheral nuclei, the malarian pigment is subperipheral in location in vacuoles of various size and the central zone of the cell is light and completely devoid of malarian pigment. These formations result from the fusion of several pigment-bearing cells at a time coincident with the disappearance of merozoites and plasmodii from the peripheral blood and with the regeneration of the normal structures of the liver. Our data suggest that the origin of the giant cells is represented by the pigment-charged Kupffer cell, dislocated from the walls of the hepatic sinusoids during the regeneration of the liver. The removal of multinucleated cells from the liver proceeds mainly by the perilobular veins.

Investigating the liver histology of malarian rodents during recovery, we observed, in addition to the rapid regeneration of the hepatic cords and cells, the occurrence of big, multinucleated cells, heavily charged with malarian pigment. The present paper is concerned with a description of these cell types and their distribution inside the hepatic lobules and in the extralobular spaces, as well as with their origin and role.

MATERIAL AND METHODS

The following *Plasmodium berghei* strains were used: strain "Copenhague" — Cantacuzino Institute 1972, strain "London" — Cantacuzino Institute, 1972, strain "Prague" — Cantacuzino Institute 1972, among which, the first displayed the lowest virulence and the latter the highest one, mortal for any non-immunized hamster and mouse. Non-immunized animals were given intraperitoneally 0.1 ml mouse or hamster blood, parasited by the strain "Copenhague" — Cantacuzino Institute 1972. Animals immunized by natural recovery were given a similar dose of blood parasited with virulent *Plasmodium berghei* strains. Immunized animals, as well as animals at the first recovery, were killed at time intervals ranging from 7 to 30 days, after the disappearance of the parasite in the blood. Rats, known to display natural resistance against *Plasmodium berghei*, were sacrificed after 30 days from the infestation with the Prague strain. 6 white mice, 8 golden syrian hamsters and 3 white rats were used, all of which were adult individuals. Large samples of hepatic tissue, originating from 2 hepatic lobes for each individual, were fixed in Helly liquid and embedded in paraffin. Histological preparations, 5 μ in depth, were stained in haemalun-eosin-orange G and May-Grünwald-Giemsa-acetic acid. The above staining procedures were also applied after permanganic oxidation, which was performed for 3 minutes in the following mixture: sulphuric acid 3%, potassium permanganate 3% and distilled water (1 :1 :6), followed by whitening in oxalic acid 3%, to reveal the structures after the removal of malarian pigment. To establish the nature of the pigment, the following histochemical reactions were performed: argentaffinic reaction according to Masson, the pigment bleaching by chloric whitening (Mayer) and the oxidation of malarian pigment with sulphuric alcohol [5].

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RESULTS

Multinucleated cells are more frequent and larger in size in th hamster liver as compared to the mouse liver. They are located in th hamster liver mainly in the peripheral zone of the hepatic lobule (Fig. 2 In the central zone, their poor occurrence is a marked difference fro the mouse liver.

The multinucleated, pigment-bearing cell is a transitory formatio occuring in the liver of malarian animals during the recovery perio Cells of this type seem to provide a mechanism for the removal of reticulo histiocytic elements outside the regenerating hepatic lobe, as well as o phagocytic debris they contain. Multinucleated cells are big cellular fo mations, spherical or ovoidal in shape, containing 2—3 to 40 nuclei a the section level (Figs 4 and 6), with an average diameter up to 50—60μ In section their general appearance is circular, with nuclei located in th peripheral zone and describing a nearly regular, often incomplete rin (Fig. 5), very close to the cell membrane. Nuclei, which are quite clos with one another, often appear partly overlapping. They are spherica or ovoidal in shape and relatively basophilic, with small nucleoli an chromatin granules located on the nuclear membrane. Nuclear basophil proportionally increases with decreasing nuclear size. Inside the cell nuclei may occur which are normal in appearance and randomly distri buted, mainly in the multinucleated cells in formation (Fig. 10), whic are small in size and with an irregular shape. Picnotic nuclei occur as well showing different degeneration stages, several of which are included i vacuoles (Fig. 4). In certain zones, 2—3 multinucleated cells appear i close contact and reciprocally dammaged, which maintain the origina location of nuclei and malarian pigment (Fig. 9).

An examination of a great number of these formations allows t conclude that the possibility exists for cells of this type to fuse (Fig. 10) with the subsequent formation of a big cell, displaying the characteristi peripheral location of nuclei (Figs 3 and 4).

The malarian pigment, as usually in great amount, describes a larg circular sector located between the nuclear ring and a central zone, gene rally devoid of pigment (Fig. 3) and most nuclei are masked by it. Suit able histochemical treatments [5] provided evidence that the black pigment of the multinucleated cells, like that of Kupffer cells, free macro phages and parasited red cells is a malarian pigment. Preparations exa mined after permanganic oxidation revealed in the pigmented zone o the cell many vacuoles containing malarian pigment and rare red cells and nuclei undergoing digestion (Figs 4 and 5). Inspite of these obser vations, no active phagocytic event, performed by these giant cells wa apparent, involving red cells or reticulo-endothelial ones, located between the hepatic cords and the multinucleated cells, except for several reciproca fusions of multinucleated cells.

The central zone of the multinucleated cell consists in a dense, rela tively homogenous cytoplasm, devoid of phagosomes (Figs 2 —5). The occurrence of red cells close to the giant cells suggests that these latte

are generally located in highly enlarged sinusoid vessels in the respective zone.

In hamster and mouse, giant cells are very frequent in the peripheral zone of the lobules and in the porto-biliary spaces, as well as close to the perilobular veins, which suggests the main mechanisms by which these formations are removed from the liver and respectively, from the lobule (Figs 1 and 2).

In mouse, hyperpigmented macrocells frequently appear to be eliminated by the centrolobular veins too. Moreover, the removal of these cells might proceed also via the connective capsule of the hepatic lobe directly in the intraperitoneal space.

After recovery, in one mouse a lobe was observed to contain many pigmented multinucleated cells undergoing elimination, while from the other lobe, this type of cells was almost completely discarded. In hamster and mouse, Kupffer cells were frequently observed to be eliminated individually among the reticular cells of the Kiernan spaces towards the portal vein. In rats after recovery, no multinucleated hyperpigmented cell was observed, the only manner by which malarian pigment is removed proceeding by individual elimination of Kupffer cells in the porto-biliary spaces.

As to the origin of the pigmented multinucleated cells, our observations seem to indicate as a starting point, the Kupffer cells which have phagocyted high amounts of parasited red cells and merozoites and which, unlike the monocytes in the sinusoid vessels, failed to be removed in the blood after the peak stage of the disease. However, the involvement of free macrophages (Fig. 8) in the generation of multinucleated cells should not be ruled out.

The removal of exhausted Kupffer cells, overcharged with malarian pigment is very necessary for the liver during regeneration, as their retroconversion to the normal type seems to be very difficult. One might assume that 2—3 neighbouring Kupffer cells (Fig. 7), dislocated together from the wall of the regenerating sinusoid vessel and subsequently undergoing the pressure imposed by a reduced sinusoid, are likely to generate a small multinucleated cell. The passive transport of these residual cells towards the elimination sites might provide opportunities for them to come in contact and to fuse with one another (Figs 9 and 10).

In conclusion, the giant multinucleated cells seem to originate as syncitial cells by serial fusion of cells or syncytes smaller in size, with the subsequent cumulation of nuclei, cytoplasm and malarian pigment, resulting in the observed structural pattern. Mitoses and nuclear budding seem to play a minor part in the generation of the number of nuclei in the multinucleated cells, as no nuclear multiplication event was encountered in the examined preparations.

Granulome-like cellular aggregates were also observed, consisting in a small number of hypertrophic pigment-bearing macrophages, distributed among the numerous lymphoid-type cells, haemocytoblasts, eosinocytes, degenerating cells etc. These granulomes are eliminated as a whole, by the same ways as pigmented multinucleated cells (Figs 11 and 12).

DISCUSSION

The giant, pigment-bearing multinucleated cells were not reported by other investigators [1] [7] [8]. Furthermore, no systematic investigation of the mammalian liver histology during recovery after malarian disease still exists.

Giant multinucleated cells are known in the histopathology of proliferative inflammations [2] [9] [10], or after the long term action of pathogenic factors upon a wide range of reticulohistiocytic cells [4] [6]. The histogenesis of these classes of reactive cells reveals either a predominantly plasmodial origin, as evolving from a repeated multiplication of nuclei without cytodieresis, or a syncytial one, consisting in the fusion of several histiocytes around the virulent bacteria, resulting in the isolation and delimitation of the infectious center inside a giant cell [9] [10].

Experimental models were used to demonstrate the effect of phospholipids which induce the formation of multinucleated epithelioid cells and of long-chain fatty acids which induce the formation of Langhans-type cells [3] [10]. Several observations indicate the possible cytodifferentiating ability of hemoglobin derivatives (hemine, protoporphyrin, hematoporphyrin), which upon administration in healthy mice induced the formation of many megakaryocytes in the liver [12] [13]. The above result lead to the temptative hypothesis that the formation of megakaryocytes is based upon the Kupffer cells, which are known to accumulate these derivatives. Other authors [11], investigating the location of hemoderivatives, insist upon the Kupffer cell too, claiming however, that the hepatocyte were also a site of noticeable pigment accumulations. Garnham [14] found and illustrated cellular clusters with malarian pigment in liver of organisms infested with *Plasmodium coatney;* it is possible that some of such cell agglomerations are giant cells dissimulated by the plentiful malarian pigment.

The present paper provides further support to the idea that the malarian pigment-bearing Kupffer cells are able to evolve, in certain conditions, to form syncytial cells. Unlike the proliferative Langhans-type cells and the megakaryocytes, the pigmented multinucleated giant cell is a syncytial formation which stores and removes the debris phagocyted by the original cell and which results from the combined action of the malarian pigment as a characteristic cytodifferentiating factor, and the pressure exerted by the regenerating liver cords to which are exposed the neighbouring pigment-bearing cells.

The reliability of these assumptions is supported by the following observations :

a) Syncytes of this type do not occur during the malarian disease, even when this latter lasts for more than 3 weeks, during which pigment accumulations proceed in many phagocytic cells due to their motility, including the Kupffer cells, which are readiliy removed by the sinusoid vessels, enlarged in this period.

b) These syncytes do not result from a passive fusion of certain cells (destroyed hepatocytes, lymphocytes, polymorphonuclear cells, monocytes and Kupffer cells), subsequent to the liver regeneration, as in this case, among the pigmented cells many other cell types occur. Such cell clusters, of the granulome type, were actually observed until their elimi-

PLATE I

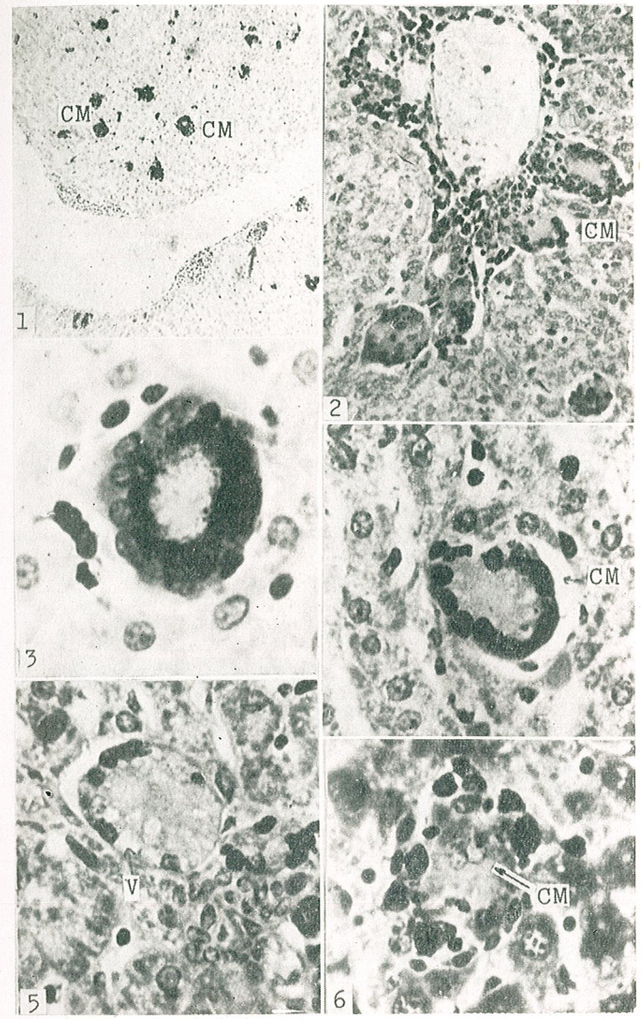

Fig. 1. — Multinucleated cells (*CM*), Kupffer cells, around a perilobular great vein (hamster).
Fig. 2. — Several multinucleated cells (*CM*) which are eliminated by porto-biliary space (hamster).
Fig. 3. — One pigment-bearing multinucleated cell.
Fig. 4. — Multinucleated cell (*CM*), with nuclei partly overlapping located in the peripheral zone ; in the middle of the cell there are vacuoles with subperipheral location and in the central zone is a dense, relatively homogenous cytoplasm (hamster).
Fig. 5. — Multinucleated cell ; there are many vacuole (*V*) in the cytoplasm (hamster).
Fig. 6. — Small multinucleated cell (*CM*), with some lymphoid-type cells and reticulo-histiocytic elements around it (mouse).
Figs 1 and 3 : hemalaun-eosin-orange G. Figs 2,4,5 and 6 : May-Grünwald Giemsa — ac. acetic, after permanganic oxidation.)

nation from the liver, but we failed to record within them any cell fusion.

c) As in rat, due to the natural resistance, the parasitic disease with *Plasmodium berghei* is poorly represented and complete recovery is spontaneously reached after 14 days, pigment-overcharged Kupffer cells are rare and eliminated one by one, while no pigmented syncytes is apparent.

REFERENCES

1. Ciucă M., Radacovici E., Ciplea A. G., Istfan T., Ianco L., Arch. Roum. Path. Microbiol. 1962, **23**, *1*, 7—22.
2. Ciurea V., *Anatomia patologică a animalelor domestice*. Ed. Didactică şi Pedagogică, Bucharest, 1971, 195—200.
3. Eskenasy A., *Imunomorfologia şi imunopatologia plăminului*, Ed. Acad. R.S.R., Bucureşti, 1972, 71—88.
4. Jaffé C. R., *Reticuloendotelial System* in : *Handbook of Haematology*, Downey Hal Ed., New York, 1938, vol. II, 1021.
5. Lison L., *Histochimie et cytochimie animales*, Ed. Gauthier-Villard, Paris, 1960, 628, 725, 769.
6. Mann F. C., Higgins M. G., *Liver histiocytes fixed system* in : *Handbook of Haematology*, Downey Hal Ed., New York, 1938, 1377.
7. Martin L. K., Einheber A., Porro R. F., Sadum E. H., Bauer H., Res. in Malaria, Suppl. in Military Med., 1966, **131**, *9*.
8. Miwa S., Tanikawa K., Rev. Internat. Hepatol., 1965, **15**, 489—496.
9. Onet E., Rec. Med. Vétér. Ec. Alfort, 1972, **148**, *9*, 1045—1052.
10. Oprescu C. C., *Infecţia cu micobacterii*, Ed. Academiei R.P.R. Bucharest, 1963, 63—73.
11. Pimstone N. B., Tenhunen R., Seitz P. T., Marver H. S., Schmid R., J. Exper. Med., 1971, **133**, *6*, 1264—1281.
12. Tverdy G., Acta haematol., 1967, **38**, *1*, 43—48.
13. — Nouvelle Rev. Franç. d'Hematol., 1968, **8**, *1*, 53—64.
14. Garnham P.C.C., *The pathology of Plasmodium coatneyi malaria* in *Omagiu Acad. Prof. Dr. M. Ciucă*, Ed. Academiei' Bucarest, 1965, 199—203.

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PLATE II

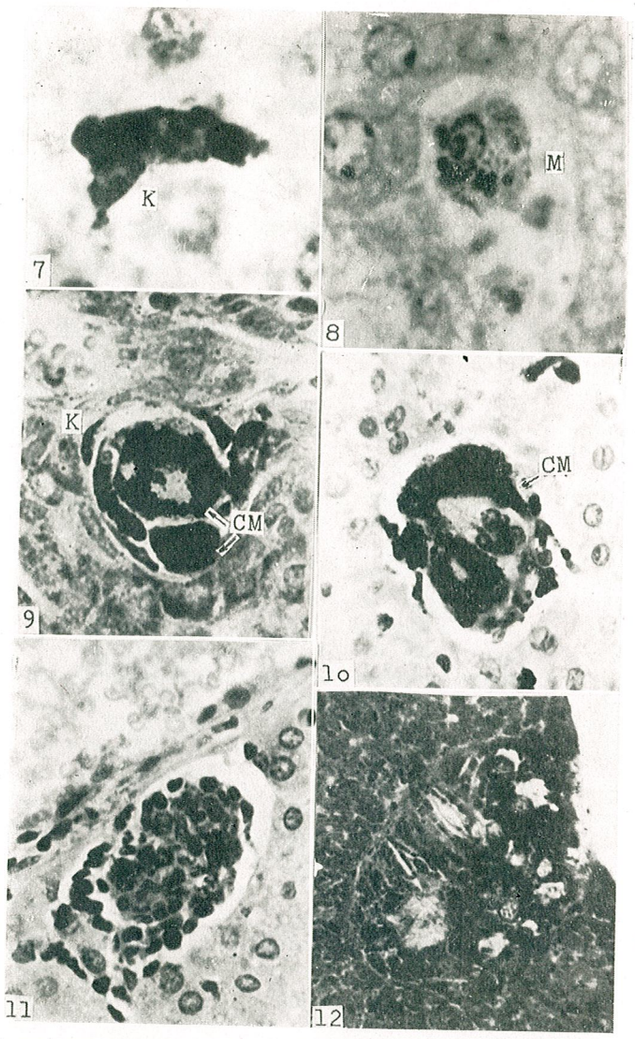

Fig. 7. Two pigment-bearing Kupffer cells (*K*), which appear to be fused (mouse).
Fig. 8. — A pigment- bearing macrophage (*M*) in a highly enlarged sinusoid vessel (mouse).
Fig. 9. — Two multinucleated cells (*CM*) and some neighbouring Kupffer cells (*K*), which eventually can fuse (hamster).
Fig. 10. — A moment after fusion of many multinucleated cells (*CM*). The nuclei and malarial pigment still keep the previous aspect of separate cells (hamster).
Fig. 11. — Higher power of a sector of figure 1 (arrow). Aggregations of different types of cells in the moment of elimination ; there is little number of pigmented phagocytic cells (hamster).
Fig. 12. — Granulom-like cellular aggregates in elimination via the connective capsule of the hepatic lobe directly in the intraperitoneal space.
Figs 7, 8, 9 and 12 : May—Grünwald Giemsa ac. acetic. Figs 10 and 11 : hemalaun—eosin—orange G.

RECHERCHES SYSTÉMATIQUES BASÉES SUR L'ÉLECTROPHORÈSE, CHEZ CERTAINS BLENNIIDAE (PISCES) DE LA MER NOIRE

PAR

LOTUS MEŞTER et CĂLIN TESIO

The electrophoretical pattern of the sarcoplasmatic proteins of four species belonging to the Family Blenniidae (Pisces) is different and specific for each species. Our results confirm the validity of the species *Blennius ponticus* Slast.

L'étude de la famille des Blenniidae s'est avérée d'un intérêt tout particulier, vu qu'une partie des espèces ont une large aire de répartition et que leur morphologie externe présente des différences dues à leur adaptation à un certain biotope. Les observations de Mayer [4] révèlent le fait que l'un des caractères morphologiques fort utilisé dans la taxonomie des Blenniidae, à savoir, le nombre des génipores du système latéral céphalique, peut varier chez les représentants d'une même espèce, collectée dans la mer Noire et la mer Méditerranée. La grande variation intraspécifique, ainsi que l'étude de certains caractères morphologiques isolés, ont soulevé des controverses concernant la validité de certaines espèces comme fut le cas de *Blennius ponticus* Slast.

C'est pour ces raisons que nous avons appliqué la méthode de l'électrophorèse dans l'étude de cette famille, afin de préciser, sur la base de certains critères, la séparation des espèces de Blenniidae. On sait que le spectre électrophorétique, surtout celui des protéines sarcoplasmatiques, est spécifique, ce qui le rend utile et efficient dans la caractérisation des espèces discutables [2] [10—12].

MATÉRIEL ET MÉTHODE

On a effectué des examens électrophorétiques des protéines sarcoplasmatiques, chez quatre espèces appartenant au genre *Blennius*: *Blennius sanguinolentus* Pall., *Blennius tentacularis* Brunn, *Blennius ponticus* Slast. et *Blennius sphynx* Val., collectées dans la mer Noire (Agigea et Eforie). On a utilisé la méthode de l'électrophorèse en gel d'amidon, d'après Smithies [9], modifiée par Mouray et collab. [6].

RÉSULTATS ET DISCUSSION

Les espèces du genre *Blennius* investiguées, présentent un modèle électrophorétique des protéines sarcoplasmatiques assez unitaire, formé de 8—12 bandes, à disposition généralement similaire.

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Chez *Blennius tentacularis* le modèle électrophorétique présente bandes, dont une à vitesse réduite (Rf 0,30), trois bandes à vitesse de migration moyenne (Rf 0,53—0,62) et les autres trois bandes protéiques vitesse semi-rapide (Rf 0,79) et rapide (Rf 0,93 et 0,99) (Fig. 1 *A*).

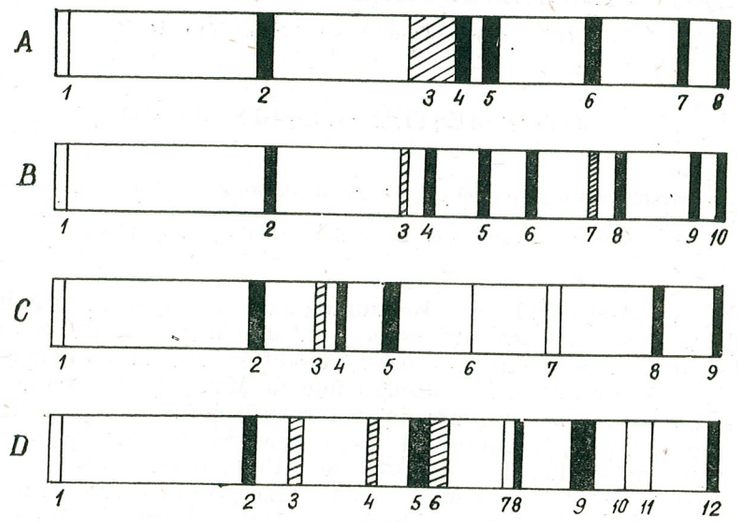

Fig. 1. — Les modèles électrophorétiques des protéines sarcoplasmatiques chez : *A*, *Blennius tentacularis* ; *B*, *Blennius sanguinolentus* ; *C*, *Blennius ponticus* ; *D*, *Blennius sphynx*.

Chez *Blennius sanguinolentus* le modèle électrophorétique des protéines sarcoplasmatiques est formé de 10 bandes, dont celle à vitesse de migration réduite a une localisation similaire à l'espèce antérieure, cependant que les autres bandes protéiques ont une répartition relativement hétérogène, vers le bout anodique du modèle électrophorétique (de Rf 0,52 à 0,99) (Fig. 1, *B*).

Blennius ponticus est caractérisé par un modèle électrophorétique des protéines sarcoplasmatiques formé de 9 bandes protéiques, dont la répartition est : un groupe de quatre fractions protéiques à vitesse de migration réduite (Rf 0,30—0,50), un autre groupe de trois fractions protéiques à vitesse de migration électrophorétique plus élevée (Rf. 0,63—0,90) et une bande protéique située à l'anode du modèle électrophorétique avec Rf 0,99 (Fig. 1, *C*).

Blennius sphynx présente le modèle électrophorétique des protéines musculaires le plus complexe de toutes les espèces étudiées. Chez cette espèce on a visualisé 12 fractions protéiques, groupées comme suit : les bandes protéiques 2,3 et 4 à vitesse de migration réduite (Rf 0,30—0,48), les fractions protéiques 5—8, à vitesse de migration moyenne (Rf 0,50—0,70), les bandes protéiques 9—11 situées à l'anode du spectre électrophorétique (Rf 0,80—0,90) et une bande protéique rapide avec Rf 0,99 (Fig. 1, *D*).

Comparant les modèles électrophorétiques chez les quatre espèces de Blenniidae, on constate une série de particularités dans leur distribution, permettant des appréciations concluantes quant à leur validité comme espèces distinctes. Des animaux similaires au point de vue génétique présentent une distribution constante de leurs protéines musculaires. Les résultats obtenus antérieurement par d'autres auteurs comme Tsuyuki [11], [12] et Lilievik [3], et par nous-mêmes [2], [10] ont mis en évidence la stabilité électrophorétique des protéines musculaires, permettant la réalisation de modèles électrophorétiques ressemblants et spécifiques chez les espèces du même genre.

La comparaison des modèles électrophorétiques des protéines musculaires obtenus par nous chez les Blenniidae révèle un plus grand rapprochement entre les espèces *Blennius sphynx* et *Blennius tentacularis*. Cette appréciation est basée sur le fait que les fractions protéiques 5 et 6 de la première, correspondent aux fractions 3 et 4 de la seconde, et la bande protéique majeure 9 de la première espèce correspond à la bande protéique majeure 6 de la seconde.

Compte tenu de certaines données morphologiques et écologiques, Zander [13] divise les espèces méditerranéennes de *Blennius* en deux grands groupes : *sphynx* et *canevae*. Dans le groupe *sphynx* est inclus aussi *Blennius incognitus* synonymisée par Mayer [5] avec *B. ponticus*. Nos données électrophorétiques mettent en évidence le fait qu'en dépit de certains traits écologiques communs, *B. ponticus* présente un modèle électrophorétique des protéines caractéristique et différent de *B. sphynx*.

Basés sur le modèle électrophorétique obtenu, nous sommes d'accord avec l'opinion de Slastenenko [8] sur l'espèce *B. ponticus*, la considérant comme bonne espèce et non pas comme une sous-espèce de *B. zvonimiri* [1] ou de *B. gattorugine* [7].

CONCLUSIONS

1. Les quatre espèces de *Blennius* ont des modèles électrophorétiques caractéristiques et spécifiques.

2. *B. ponticus* a la valeur d'une espèce distincte et son modèle électrophorétique est plus rapproché de celui de *B. tentacularis*.

3. Toutes les espèces du genre ont en commun les fractions protéiques à vitesse de migration réduite (Rf 0,30) et rapide (Rf 0,99).

BIBLIOGRAPHIE

1. BĂNĂRESCU P., Fauna R.P.R., Pisces-Osteichthyes, 1964, **13**.
2. GROSSU A., MEȘTER L., TEȘIO C., Trav. Mus. Hist. Nat. « Gr. Antipa », 1971, **11**, 339.
3. LILIEVIK H. A., SCHLOEMER C. R., Science, 1961, **134**, 2042.
4. MAYER R. F., Journées ichthyol., Rome, CIESM, 1970, 75.
5. — Trav. Mus. Hist. Nat. « Gr. Antipa », 1974, **15**, 315—319.
6. MOURAY H., MORETTI J., FINE J. M., Bull. Soc. Chim. biol., 1961, **43**, *78*, 993.

7. Rass T., Ihtiofauna Cernogo moria i eio ispolzovanie, Tr. Inst. Okeanol. Akad. URSS,
8. Slastenenko E. P., Bull. Soc. Zool. France, 1966, **90**, *5—6*, 541.
9. Smithies O., Biochem. J., 1955, **61**, 629.
10. Tesio C., Meşter L., Journées ichthyol., Rome, CIESM, 1970, 83.
11. Tsuyuki H. E., Roberts E., Vanstone W. E., Markert J. R., J. Fish. Res. Bd. Canada, 1965, **22**, *1*, 215.
12. Tsuyuki H., Roberts E., J. Fish. Res. Bd. Canada, 1966, **23**, *1*, 101.
13. Zander D., Rapp. Comm. Int. Mer Médit., 1974, **22**, *7*, 57.

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Bucarest 35, Splaiul Independenţei 93—95

L'APPORT DE L'ÉLECTROPHORÈSE EN GEL D'AMIDON À LA SYSTÉMATIQUE DU GENRE *MESOCRICETUS* NEHRING (RODENTIA, CRICETIDAE)

PAR

CĂLIN TESIO

Based on the electrophoretic models in starch gel of the sarcoplasmatic proteins, the validity of the species *Mesocricetus brandti* and *M. newtoni* as subspecies of *Mesocricetus auratus* is discussed.

La taxonomie des espèces de rongeurs qui appartiennent au genre *Mesocricetus* a été très discutée. Ainsi les anciens chercheurs [5] [6] les considèrent comme des espèces distinctes des différentes populations. Ellerman et Morrison-Scott [1] soutiennent que ce genre est formé d'une seule et même espèce, *M. auratus* avec de nombreuses sous-espèces. Par contre, Vinogradov et Gromov [11] admettent l'existence de deux espèces : *M. raddei* (avec les sous-espèces *raddei, avaricus* et *nigricolis*) et *M. auratus* (avec les sous-espèces *auratus* et *brandti)*, mais leurs études ne portent pas sur les populations de la Dobroudja.

Marcheş [4] dans un étude anatomo-morphologique, biologique et écologique concernant la population des hamsters de cette région de notre pays met en évidence son appartenance à l'espèce *M. newtoni* Nehr.

Cette affirmation a été soutenue aussi par les études d'écologie [3], de caryologie et biochimie du sang [7] [8] et par les tentatives d'hybridation avec *M. auratus* [9].

Dans ce travail nous nous proposons de résoudre les problèmes concernant la taxonomie de quelques espèces de *Mesocricetus* en employant les modèles électrophorétiques en gel d'amidon des protéines sarcoplasmatiques.

MATÉRIEL ET MÉTHODE

Les déterminations ont été faites sur 25 exemplaires appartenant à 2 genres et 4 espèces de la famille Cricetidae :

Mesocricetus auratus Waterhause — 5 exemplaires
Mesocricetus newtoni Nehr. — 5 exemplaires
M. brandti Nehr. — 5 exemplaires
Phodopus sungorus Pallas — 5 exemplaires

Les animaux proviennent de l'élevage du Laboratoire de génétique de la Faculté de biologie de Bucarest et ont été mis obligeamment à notre disposition par M. le Prof. P. Raicu.

Les modèles électrophorétiques des protéines sarcoplasmatiques ont été obtenus par électrophorèse en gel d'amidon d'après une méthode antérieurement décrite [2].

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RÉSULTATS ET DISCUSSIONS

Les modèles électrophorétiques des protéines sarcoplasmatique obtenus chez les représentants du genre *Mesocricetus* étudiées présente 12 à 13 bandes d'intensités et vitesses de migration différentes.

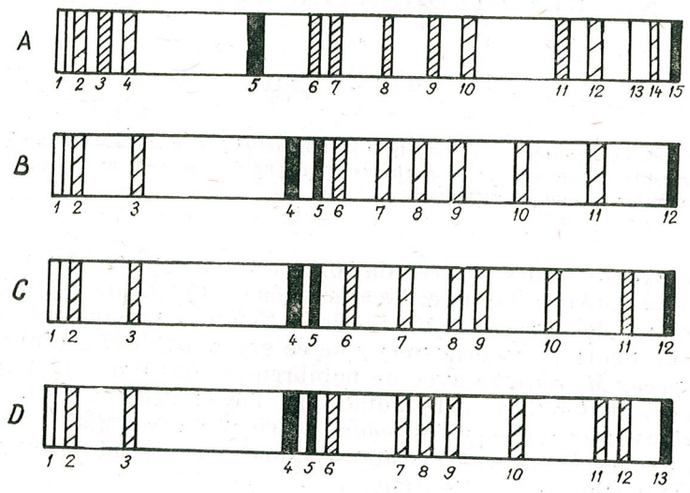

Fig. 1. — Modèle électrophorétique des protéines sarcoplasmatiques en gel d'amidon chez : *Phodopus sungorus* (*A*) ; *Mesocricetus brandti* (*B*) ; *M. newtoni* (*C*) ; *M. auratus* (*D*).

L'aspect général de modèles est très semblable ayant 8 bandes communes (Fig. 1 *B—D*). Ce fait nous indique une proche parenté de ces espèces, ce qui nous suggère que la spéciation chez le genre *Mesocricetus* est relativement récente.

Quand même, il faut souligner que les modèles sont différents les uns des autres, fait que nous permet donc de soutenir qu'en réalité il s'agit d'espèces bien distinctes. Chez chaque espèce le modèle obtenu est très constant n'étant pas sujet aux influences directes ou indirectes des conditions de milieu. Des variations ont été signalées seulement dans le cas de dystrophie musculaire [10] mais dans notre déterminations nous n'avons employé que des exemplaires sains. À titre de comparaison, nous avons fait des déterminations électrophorétiques chez *Phodopus sungorus* où le modèle présente 15 bandes (Fig. 1, *A*) dont la disposition est bien différente de celle des modèles obtenus du genre *Mesocricetus*. Toutefois les modèles électrophorétiques chez les deux genres mentionnés ont 5 bandes communes (bandes 1, 2, 4, 6, 10 de *Phodopus* qui correspondent aux bandes 1, 2, 3, 5, 9 de *Mesocricetus*).

CONCLUSIONS

— Le modèle électrophorétique des protéines sarcoplasmatiques chez ces rongeurs est caractéristique et en même temps constant pour chaque

espèce étudiée et en offrant en plus, la possibilité de mettre en évidence le groupement des espèces dans des genres.

— L'étude des électrophorégrames obtenus nous permet de soutenir la validité des espèces *M. brandti* et *M. newtoni* et de rejetter l'acception de certains auteurs, selon laquelle elles seraient des sous-espèces de *M. auratus*.

— En même temps, nos résultats basés sur l'électrophorèse en gel d'amidon des protéines sarcoplasmatiques nous font penser à une différentiation relativement récente des espèces au sein du genre *Mesocricetus* ce qui expliquerait en grande partie les contradictions des spécialistes quant à la systématique de ce genre.

BIBLIOGRAPHIE

1. Ellerman I. R., Morrison-Scott T. C. S., *Checklist of Palearctic and Indian Mammals 1758 to 1949*, British Museum, London, 1951.
2. Grossu Al. V., Meşter L., Tesio C., Trav. Mus. Hist. Nat. „Gr. Antipa", 1971, **11**, 319—346.
3. Hamar M., Şutova M., Z. f. Säugetierkunde, 1966, **31**, *3*, 237—251.
4. Marcheş G., St. Cerc. Igienă şi Sănătate Publ., 1964, 185—213.
5. Nehring A., Zool. Anzeiger, 1898, **21**, 329—332.
6. — Zool. Anzeiger, 1898, **21**, 493—495.
7. Raicu P., Bratosin D., Z. f. Säugetierkunde, 1966, **31**, *3*, 251—255.
8. Raicu P., Hamar M., Bratosin S., Borşan I., 1968, **33**, *2*, 186—192.
9. Raicu P., Ionescu-Varo M., Duma D., J. of Heredity, 1969, **60**, *3*, 149—152.
10. Schapira G., Dreyfus I. G., C. R. Soc. Biol. Paris, 1958, **152**, *12*, 1705—1707.
11. Vinogradov R. S., Gromov I. M., *Grizuni faunt S.S.S.R.*, Izdat. Akad. Nauk. S.S.S.R., Moskva, 1952.

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PONDERAL MODIFICATIONS IN CHICKENS WITH HYPOTHALAMIC LESIONS INDUCED BY L-MONOSODIUM GLUTAMATE

BY

CONSTANȚA MATEI-VLĂDESCU, G. APOSTOL, VALERIA RĂDĂCEANU-ARICESCU and ELISABETA IONESCU

Cornish male chickens which were given an oral dose of 2 mg MSG/g b.w. daily, between the 3rd and the 10th day after hatching, presented at the age of 9 weeks an average body weight higher than that of control chickens or of those treated with a single dose of 2 mg MSG/g b.w. on the third day of life.
In the hypothalamus of these chickens degenerative changes were found at the level of tuberal nuclei. The population of neurons in the area of these nuclei appeared markedly decreased.

★

The selective hypothalamic damaging effect of L-monosodium glutamate (MSG), evidenced by Olney [9—11] in infant animals of several species of mammals, was also evidenced in chickens [16].

In broilers which received MSG during the first days after hatching no higher weight gain, as described by Olney [9] in mice, was however recorded. It should be noted that after several months the broilers thus treated were heavier than the controls of the same age, the weight differences not being, however, statistically significant [3] [6].

Working on small lots of broilers (10—15 individuals) which had a considerable individual variability of growth rate and weight gain heritabilities given their hybrid character, it is possible not to have been able to reveal a statistically significant effect on gain in weight. We therefore considered of interest to study the effects of MSG on Cornish chickens which have more homogenous phenotypic characters.

MATERIAL AND METHOD

The experiments were performed on 92 Cornish sexed chickens (46 males and 46 females). The chickens were brought into the laboratory at the age of one day. Up to the age of two weeks they were grown in common in a simple stockfarm consisting of a dark and heated compartment (19—30°C) and a bright and semi-heated one (21—24°C). Then up to the end of the experiments (63 days), the chickens were separated into lots and were put into cages, two to three chickens to a cage. The cages were maintained at room temperature, in conditions of continuous lighting. The food consisted of concentrated fodder for meat chickens.

Experiments were performed on three chicken lots, a control and two experimental ones. Each lot was made up of 24 chickens (12 males and 12 females). The chickens of the control lot were not subjected to any kind of treatment. Those of the first experimental lot were treated with a single 2 mg MSG/g b.w. dose on the third day after hatching. The chickens of the second experimental lot were given, as from the 3rd to the 10th day of life, a daily dose of 2 mg MSG/g b.w. each. A 2 mg dose was chosen as we had found in our previous experiments on broilers, that this dose had had a certain favourable effect upon weight gain and food intake [6].

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Chemically pure L monosodium glutamate ($C_5H_8NNaO_4 \cdot H_2O$) supplied by Merck. M in a 10% aqueous solution was administered by an esophagial sound.

The evolution of the body weight was recorded weekly. For this purpose, the chickens p viously submitted to a 16—18 hours fast, were individually weighed on the last day of e week. As from the 5th week the quantity of feed consumed by chickens during the following 5 a respectively 24 hours after the weekly weighing was measured. From the data obtained avera weekly weight gain, relative biweekly growth rate $\left(Vr = \frac{M_2 - M_1}{M_1} \cdot 100 \right)$, average grow rate for 9 weeks $\left(VM = \frac{M_2 - M_1}{\frac{M_2 + M_1}{2}} \cdot 100 \right)$, feed intake per individual and per kg bo weight, in 5 and 24 hours respectively, were calculated. The data were statistically p cessed, the probability of the standard error being established, by the application of the " Student's test.

For the histological control of the brain 12 chickens treated with MSG and 8 cont chickens were sacrified. The brains taken immediately after the decapitation of the chicke were fixed. Three types of fixators were used: Bouin, Stive and neutral formalin. The inclusi was made in paraffin and the sectioning at the microtome at 8—10 microns thickness. Thr staining techniques were tried: Azan, haematoxylin-eosine, and cresyl violet [7]. The be suited proved to be the haematoxylin-eosine staining.

RESULTS

The results concerning the weekly evolution of the body weigh per sexes in the three lots are presented in the graphs of figure 1. As i can be seen, the average live weight of the chickens which received MSG particularly in repeated doses, is higher than that of control chickens In females, these differences are small and statistically significant. I males, the differences are larger and statistically significant ($p < 0.01$) Thus the difference recorded at the end of the 9th week, between th average body weights of control end repeatedly with MSG treated cock was of 309.13 g. Likewise a significant weight difference ($p < 0.02$) o 257.89 g was found, between the cocks of the lot with repeated MS treatment and those of the lot which received a single MSG dose.

Analysing the evolution of the average weekly weight gain (Fig. 2 it is found that the highest weekly gain were realised by the males which received repeated doses of MSG.

In graphs of figure 3 the percentual values of the relative biweekly growth rate are given. In the case of MSG administration, in chickens of both sexes, a relative growth acceleration can be observed at the 0—2 week period of age. Subsequently, the relative biweekly growth rate o treated with MSG female chickens is very close to that of control female chickens. In treated males, beginning from the 4th week, a certain increase of the relative biweekly growth rate is again observed.

The average growth rate for the entire period of 9 weeks was slightly increased in chickens which were administered repeated MSG doses.

The followed two figures (Figs 4 and 5) comprise a series of graphs which illustrate the values of feed intake per individual and per Kg b.w., on period of 5 and respectively 24 hours. In the case of females, values were found fairly close to the three lots (Fig. 4 *B*, *D*; fig. 5 *B*, *D*). In

males, feed consumption per individual was greater in chickens of the lot repeatedly treated, being given their higher body weight (Fig. 4 *A*, *C*). Related to 1 kg b.w. feed intake was however, always smaller in cocks which received glutamate (Fig. 5 *A*,*C*).

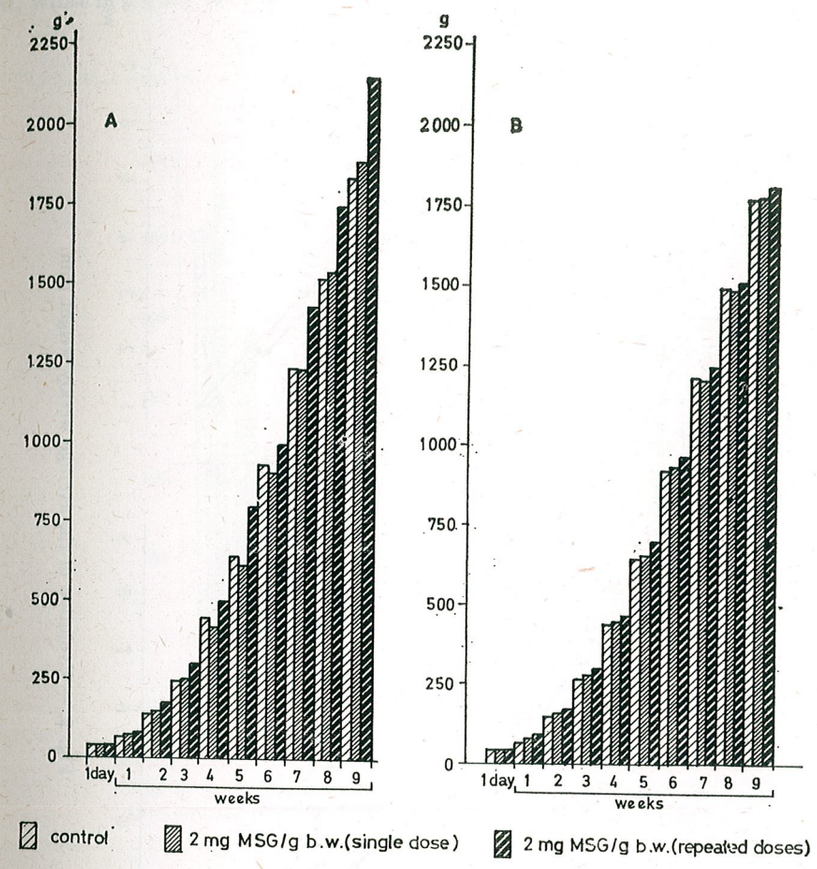

Fig. 1. — Evolution of body weight.

Microscopic examination of brain sections evidenced typical degenerative modifications in the basal hypothalamic region in chickens treated with MSG. The most striking changes appeared on the both side of the infundibular recess (Fig. 6), where in conformity with the stereotaxic atlas of Van Tienhoven and Juhasz [17] and with Völker's descriptions [18] the tuberal nuclei are to be found.

In normal individuals a grouping of small neurons is observed in this area placed very close to one another (Fig. 7). This same area appears edematized at 5 hours after oral administration of a single 2 mg

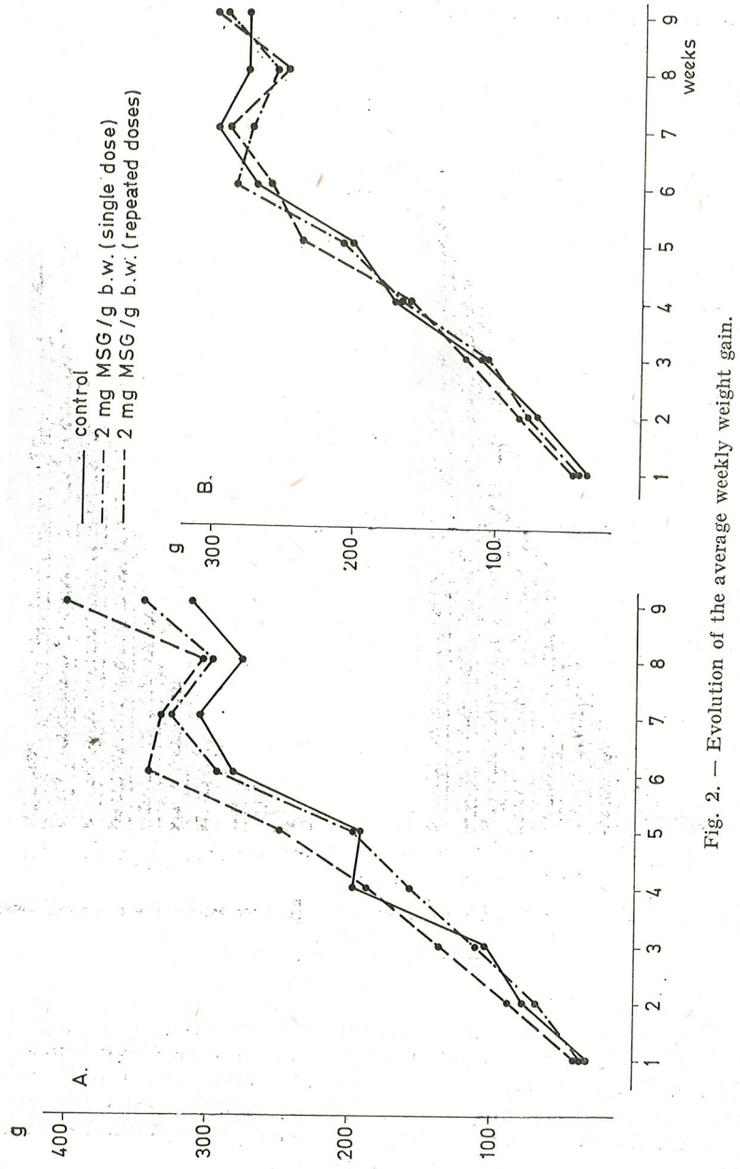

Fig. 2. — Evolution of the average weekly weight gain.

MSG/g b.w. dose. Many neurons are necrotic with bloated cytoplasm and pyknotic nuclei (Fig. 8). Degenerative changes are still more evident after 24 hours from the administration of the 8th daily dose of 2 mg MSG/g b.w. (Fig. 9). Edema persists and at the same time numerous dense, dark, bodies of variable dimensions and shapes, many vacuoles and, what is highly characteristic, a small number of neurons are observed.

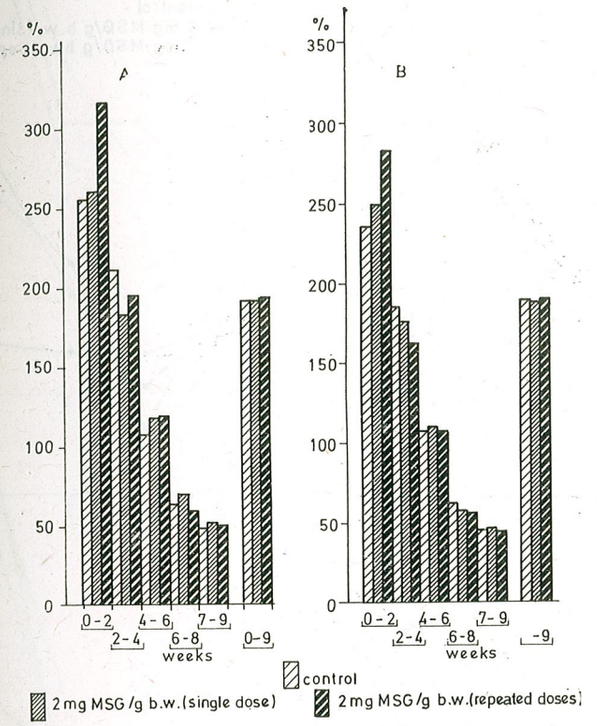

Fig. 3. — Percentual values of the relative biweekly growth rate and the average growth rate on the entire 9 week's period.

DISCUSSION

The results obtained by us in Cornish chickens, particularly in male chickens, plead for the existence of a stimulating influence of L monosodium glutamate on gain in weight. As the analysis of the relative biweekly growth rate shows, stimulation takes place in the first fortnight of age, in the period of MSG administration. An acceleration of the growth rate and a significant improvement of food conversion efficiency were likewise shown by Shannon and co-workers. [14] in male broilers, which as from the age of 7 days received diets supplemented with 12 % glutamic acid, during 11 days. This makes us consider that the stimulation of

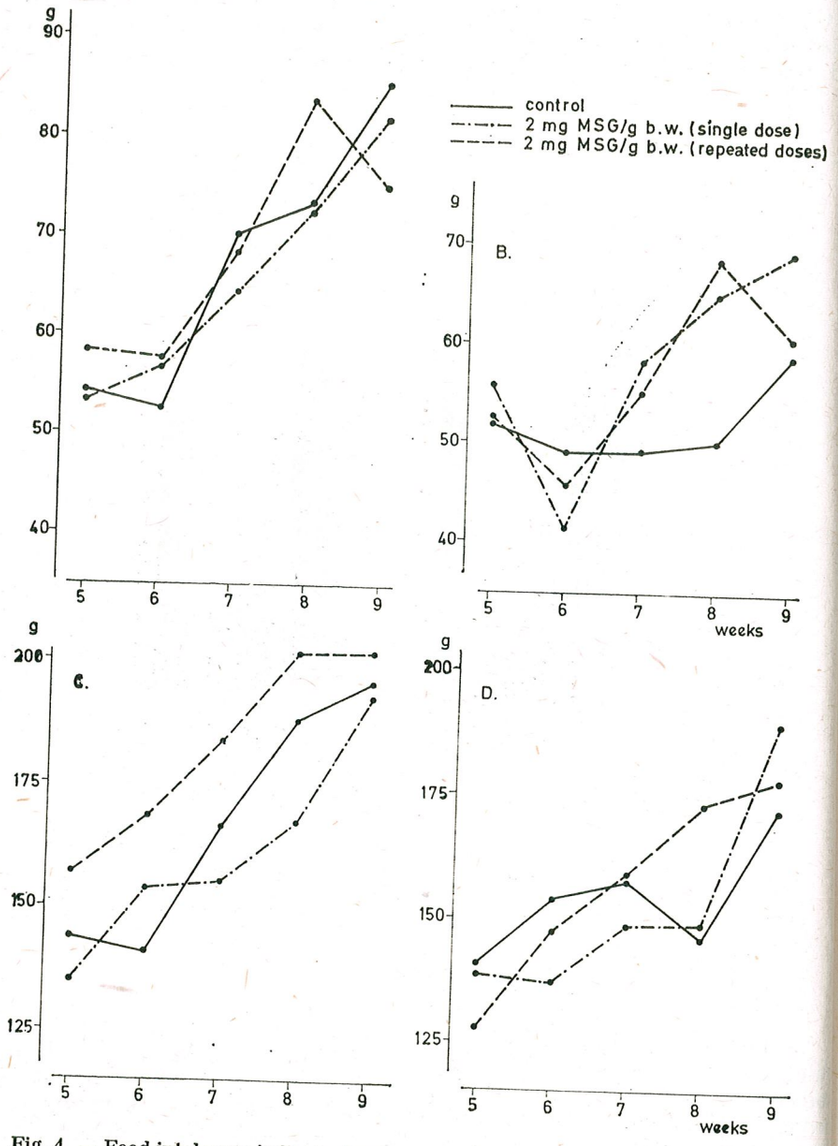

Fig. 4. — Food intake per individual over a period of 5 (*A*, *B*) and respectively 24 hours (*C*,*D*). *A*, *C* in male chickens; *B*, *D* in female chickens.

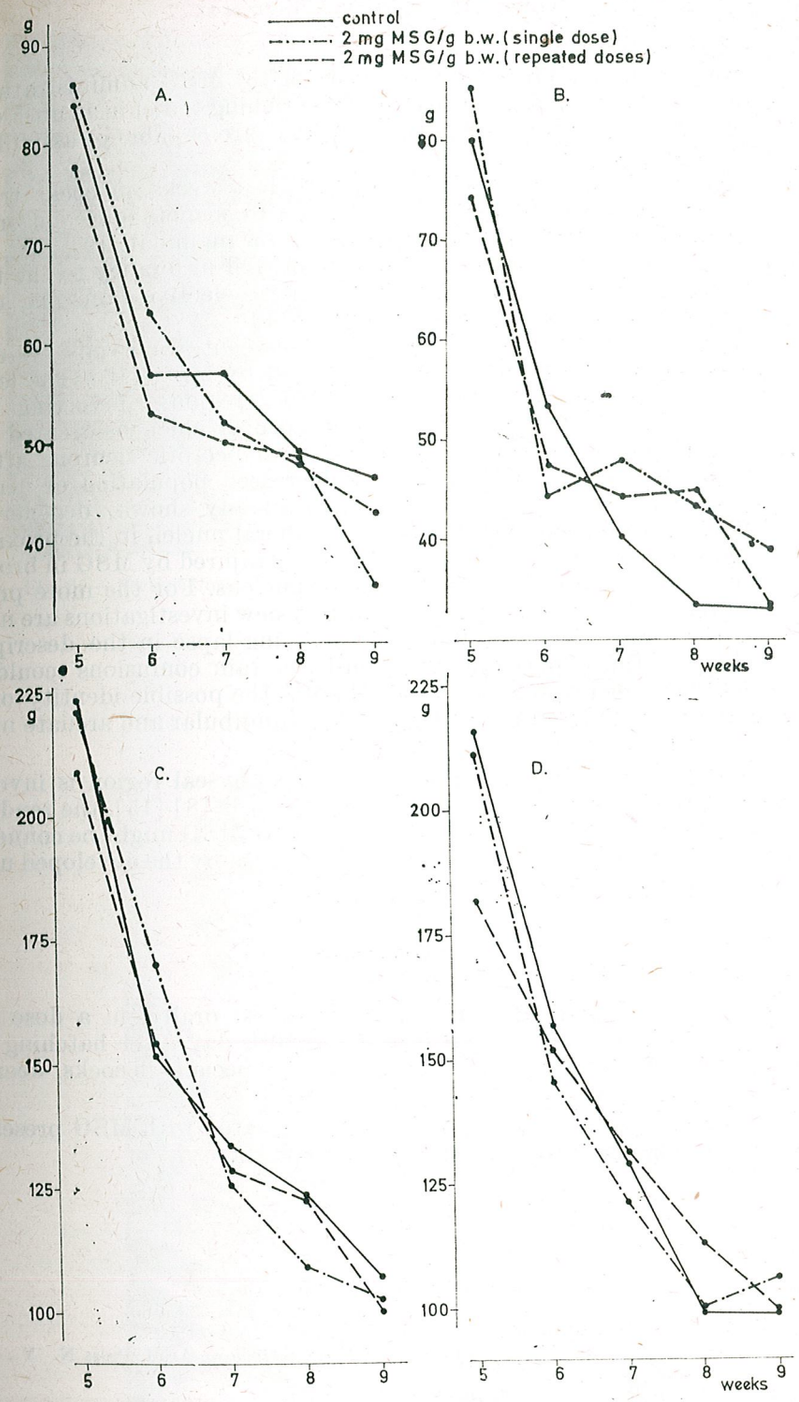

Fig. 5. — Food intake per kg body weight, on periods of 5 (*A*, *B*) and respectively 24 hours(*C*, *D*). *A*, *C* in male chickens; *B*, *D* in female chickens.

gain in weight observed by us in the period of the MSG administration, chickens of both sexes, would be due to the enriching of diet in an unessent amino acid. As mammals [20], the chickens are capable of assimilati the L aminonitrogen [12].

Higher weight gains obtained in the last weeks in cocks treat with repeated doses of MSG, might be due to a metabolic and/or ho monal imbalance. As shown by experiments on mammals [9] [13], th an imbalance would appear in animals submitted in infancy to the trea ment with high doses of MSG, a substance with selective damaging acti on certain hypothalamic nuclei [1] [2] [9—11].

Not long ago it was proved that also the central nervous system chickens (broilers) is susceptible to be injured by MSG [16]. The lesio are produced also in the hypothalamic region, involving t arcuate nuclei. In the hypothalamus of Cornish chickens treated wi MSG, we also found oedematized areas with necrotic neurons after single dose of MSG, or with markedly decreased population of neuro after 8 daily doses of MSG. As we have already shown, degenerativ modifications were located at the level of tuberal nuclei, in the proximit of the region indicated by Snapir [16] as being injured by MSG in broiler a region in which he includes the arcuate nucleus. For the more precis delimitation of the nuclei submitted to injury new investigations are neces sary, all the more so as, due to certain existing lapse in the descriptiv terminology of the hypothalamus in birds, certain confusions could b made [5]. Thus for instance Frankel [5] notes the possible identity of th tuberal nuclei of Wingstrand [19] with the infundibular and arcuate nucle of Farner et al. [4].

As it is considered that the tubero-hypophyseal region is involve in the control of the reproductive function in birds [8] [15], the tendenc towards higher weight gains in cocks treated with MSG might be connecte to the modification of the gonadal function induced by the developed unde the action of MSG tuberal lesions.

CONCLUSIONS

1. L monosodium glutamate, administred orally, in a dose of 2 mg/g b.w., daily, between the 3rd and the 10th day after hatching can stimulate gain in weight in Cornish chickens especially in cocks, decreasing at the same time feed intake per kg b.w.

2. The hypothalamus of chickens thus treated with MSG presented degenerative changes at the level of tuberal nuclei.

REFERENCES

1. Arees E. A., Mayer J. Science, 1970, **170**, *3957*, 549—550.
2. Burde R. M., Schainker B., Kayes S., Nature, 1971, **233**, *5314*, 58—60.
3. Carew L. B., Foss D. C., Poultry Science, 1971, **50**, *5*, 1501—1502.
4. Farner D. S., Wilson P. E., Oksche A., *Neuroendocrinology*, Acad. Press N. Y., 1967, **2**, 529—582.
5. Frankel A., Poultry Science, 1970, **49**, *4*, 869—911.

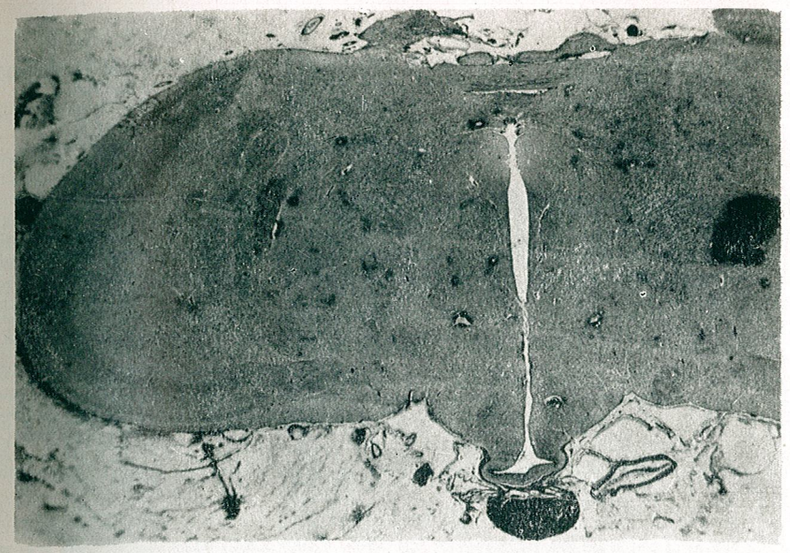

Fig. 6. — Section through chicken hypothalamus at the level of tuberal nuclei.

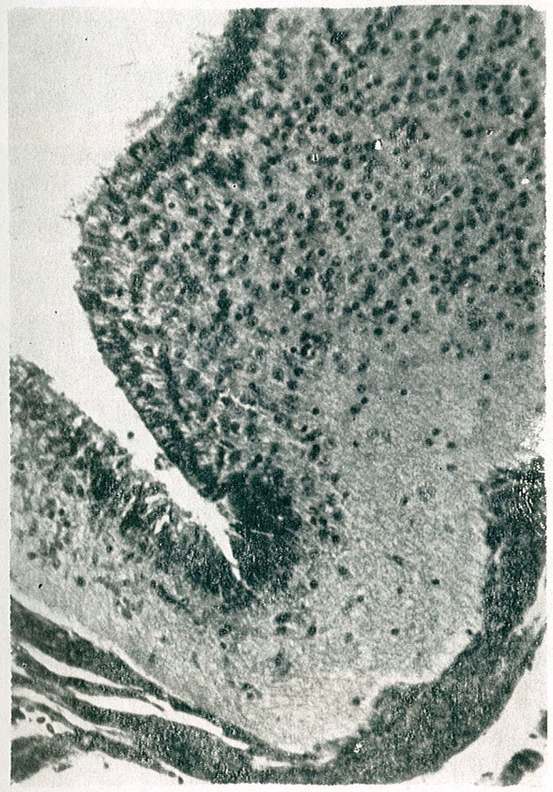

Fig. 7. — Aspect of tuberal nucleus in a control sample.

Fig. 9. — Degenerative modifications in the area of tuberal nucleus at 24 hours from the oral administration of the 8th daily dose of 2 mg MSG/g b.w.

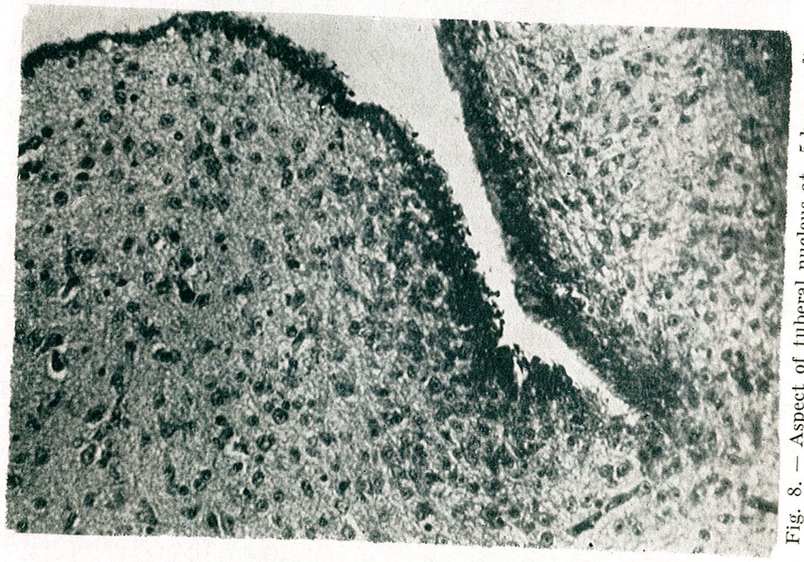

Fig. 8. — Aspect of tuberal nucleus at 5 hours after the oral administration of 2 mg MSG/g b.w,

6. Matei-Vlădescu Constanța, Apostol Gh., Barbu N., Ionescu Elisabeta, Radaceanu-Aricescu Valeria, St. și cerc. biol., Seria Zoologie, 1973, **25**, *3*, 233—244.
7. Mc. Manus J. F. A., Mowry R. W., *Staining methods. Histologic and Histochemical.* Harper & Row, N. Y., 1960.
8. Oemke H. J., Erg. Bd. Anat. Anz., 1971, **128**, 161—163.
9. Olney J. W., Science, 1969, **164**, *3880*, 719—721.
10. Olney J. W., OI-LAN-HO, Nature, 1970, **227**, *5258*, 609—610.
11. Olney J. W., J. Neuropath. exp. Neurol., 1971, **30**, *1*, 75—91.
12. Peterson D. W., Bond D. C., Longbehn L. L., Federation Proc., 1971, **30**, 232.
13. Redding T. W., Schally A. V., Arimura A., Wakabayashi J., Neuroendocrinology, 1971, *8*, 245—255.
14. Shannon D. W. F., Blair R., MC. NAB J.M., LEE D. J., Proc. Nutr. Soc. 1970, **29**, *2*, 23 A.
15. Sharp P. S., Follett B. R., Neuroendocrinology, 1969, **5**, *3/4*, 205—218.
16. Snapir N., Robinzon B., Perek M., Poultry Science, 1971, **50**, *5*, 1511—1514.
17. Van Tienhoven A., Juhasz P. L., J. Comp. Neurol., 1962, **118**, *1*, 185—197.
18. Volker H., Anat. Anz., 1971, **129**, 159—179.
19. Wingstrand K. G., *The structural and development of the avian pituitary*, G.W.K. Gleerup, Lund, 1951.
20. Womack M., Proc. Soc. Exp. Biol. & Med., 1971 , **136**, *3*, 940—941.

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AGE-DEPENDENT ANTIINSULIN EFFECT OF HYDROCORTISONE HEMISUCCINATE UPON THE *IN VITRO* GLUCOSE UPTAKE OF RAT EPIDIDYMAL ADIPOSE TISSUE

BY

J. MADAR, NINA ŞILDAN and EUGEN A. PORA

The *in vitro* antiinsulin effect of hydrocortisone hemisuccinate (0.26 μmole/ml) was evaluated on the basis of the insulin-induced glucose uptake by the adipose tissue of various age-groups of rats. Insulin and glucose were used in concentrations of 10^{-3} units/ml and 16.7 μmole/ml, respectively.
The results suggest that the ability of the glucocorticoid to exhibit antiinsulin effect is mainly influenced by the age of tissue-donor animals.

Elsewhere we reported that during intravenous glucose tolerance test [5] [6] and insulin hypoglycemia test [8] hydrocortisone hemisuccinate in white rats exhibits a rapid age-dependent antiinsulin effect upon the glucose penetration from the blood into the tissues. Our more recent evidences pointed out that the *in vitro* sensitivity of rat epididymal adipose fat pad to insulin is markedly influenced by the age of animals [7]. It is on this basis that we have studied the *in vitro* influence of hydrocortisone hemisuccinate on the insulin induced glucose uptake by epididymal adipose tissue from various age-groups of white rats.

MATERIALS AND METHODS

For the experiments 45-, 65-, 95- and more than 365-day-old (i.e. weighing 100±2.1, 151±2.5, 219±5.8 and 420±7.2 g, respectively) male albino Wistar rats from the stockfarm of our laboratory were used.

The animals were maintained under standard environmental and dietetic conditions, fed with a dry Larsen diet in which 60% of calories were supplied by carbohydrates, 25% by proteins and 15% by fats, drinking water being provided *ad libitum*.

Before the experiments the animals were fasted 18 hours, and killed by cervical dislocation and exsanguinated by decapitation. Three equal pieces (approximately 80—100 mg each) from an epididymal fat pad were quickly excised after decapitation and incubated simultaneously in special incubation flasks, in a final volume of 1 ml Krebs-Henseleit bicarbonate buffer, containing 16.7 μmole glucose, 2 mg calf-skin gelatine, 10^{-3} units insulin, and 10^{-3} units insulin plus 0.26 μmole hydrocortisone hemisuccinate, respectively. For this purpose 6-fold recrystallized glucagon-free ox-insulin ("ORGANON", potency 25 i.u.) and hydrocortisone hemisuccinate ("BIOFARM") were used.

The prepared flasks were gassed for 10 minutes with O_2 and CO_2 (95%: 5%), and the incubation was carried out at 37.6°C in the water bath of Warburg apparatus (type "LABOR") with a shaking velocity of 90 oscillations per minute and with an amplitude of 5 cm, for 2 hours.

The initial and final concentration of glucose in the medium was determined by the colorimetric method of Nelson [13].

The results are expressed as μmole glucose uptake per 100 mg wet tissue per 2 hours, and the sensitivity of tissues to insulin as well as the antiinsulin effect of hydrocortisone hemisuccinate were evaluated by the pair method, after substracting the basal glucose uptake of tissue

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pieces (incubated without insulin) from that of their corresponding pairs incubated in the p
sence of insulin, and insulin plus hydrocortisone hemisuccinate, respectively.

The data are compared according to the Student *t* test, P values of 0.05, or less, are c
sidered statistically significant.

RESULTS

As can be seen from the data presented in table 1, the basal gluco uptake by the tissue of 45-day-old rats averaged to 3.45 μmole. In co parison with this, the basal glucose uptake in all other age-groups w markedly decreased.

Insulin, in the fat pads of every age-group significantly stimulat the glucose penetration. The insulin sensitivity of the tissues varied dire proportionally with their basal glucose uptake. The highest insulin sen tivity was found for the fat pads of 45-day-old animals, in which insul stimulated the glucose uptake with 5.05 μmole over the basal values. compared to this, the stimulatory effect of insulin for the tissues of 6 95- and more than 365-day-old rats was markedly less.

Table 1

***In vitro* glucose uptake by the epididymal adipose tissue of various age groups of white rats. B = Basal glucose uptake; I = glucose uptake / in the presence of insulin (10^{-3} units/ml); I + HC = glucose uptake in the presence of insulin (10^{-3} units/ml) and hydrocortisone hemisuccinate (0.26 μmole/ml).**

Age of animal (days)	Glucose uptake μmoles/100 mg wet weight tissue/2 hours				
	B	I	I+HC	I−B	I+HC−B
45 (16)	3.45±0.38	8.50±0.52	7.85±0.59	5.05±0.57	4.40±0.72
				P>0.25	
65 (18)	1.85±0.23 P<0.001	3.92±0.26 P<0.001	2.31±0.21 P<0.001	2.07±0.25 P<0.001	0.46±0.26 P<0.001
				P<0.001	
95 (12)	1.10±0.19 P<0.001	2,94±0.30 P<0.001	2.64±0.29 P<0.001	1.84±0.31 P<0.001	1.54±0.33 P<0.01
				P>0.50	
>365 (14)	1.97±0.24 P<0.01	4.76±0.51 P<0.001	4.23±0.42 P<0.001	2.79±0.28 P<0.01	2.06±0.24 P=0.02
				P < 0.05	

Values in the columns represent mean ± standard error of the mean. All the data are compared to the reference value (group of 45-day-old rats), while the effect of insulin and of hydrocortisone are compared within every age group. The number of experiments are given in parantheses.

At the same time, it is evident that hydrocortisone hemisuccinate had no antiinsulin effect upon the fat pads of 45-day-old rats upon which insulin exhibited its greatest effect. On the tissues of 65-day-old animals, where the sensitivity to insulin was significantly lower than in 45-day-old animals, the insulin induced glucose uptake was reduced by the glucocorticoid from 2.07 to 0.46 μmole (i.e. by 77.6 %, $P < 0.001$).

Of interest is the fact that in the case of 95-day-old animals, though their tissues exhibited the lowest insulin sensitivity, hydrortisone had no antiinsulin effect. On the contrary, in the case of more han 1-year-old animals, where the insulin sensitivity was somewhat igher, the antiinsulin effect of the steroid was obvious ($P < 0.05$).

DISCUSSION

The age-dependency of the insulin sensitivity of adipose tissue bserved here is in a good agreement with the evidences of Dobrzansky 1], Katzen [2], and Katzen and Schimke [3] who demonstrated a reater activity and quantity of the insulin sensitivity determining type I hexokinase in the epididymal fat pads of young rats than in those of dult ones. Katzen [2] and McLean et al. [9] have found that the activity of this enzyme in the adipose tissue of white rats is mainly affected y the insulin secreting capacity of endocrine pancreas. Our earlier experments performed *in vivo* indicated a higher insulin secretion in young mmature rats, in response to hyperglycemia stimulus, than in adult r old animals [4].

Munck [10—12] suggested that the epididymal adipose tissue of hite rats is one of the direct targets for the action of glucocorticoids. s results from the present data, the antiinsulin effect of hydrocortisone emisuccinate was obvious only upon the tissues of 65 - and more than -year-old rats although the sensitivity of their adipose tissues was quantiatively different. In the case of 45- and 95-day-old rats, of which tissue's nsulin sensitivity was very high or very low, respectively, the glucocoricoid had no antiinsulin activity. These differences lead us to the conclusions that between the insulin sensitivity of adipose fat pad and the ntiinsulin effect of hydrocortisone hemisuccinate upon this target tissue here is not a direct relationship, and that the age of animals is an important determining factor in the *in vitro* antiinsulin effect of this glucocorticoid. From this point of view, the present data are consistent with the bservations of Palmer [14], who pointed out that the age of white rats nfluences not only the amplitude of metabolic response to glucocorticoids, ut also the type of the response obtained. This conclusion is supported y the present data and by our earlier *in vivo* evidences, showing that the ge of white rats changes the antiinsulin effect of hydrocortisone hemisucinate, leading to quantitatively different actions upon the glucose penetration from the blood into the tissues during intravenous glucose tolerance test [6] and during insulin hypoglycemia test [8].

REFERENCES

1. Dobrzansky T., Diabetes, 1966, **15**, 430—431.
2. Katzen H. M., *Advances in Enzyme Regulation*, Ed. G. Weber, Pergamon Press, 1967, 5, 325—356.
3. Katzen H. M., Schimke R. T., Proc. nat. Acad. Sci. Wash., 1965, **54**, 1218—1225.
4. Madar J., Pora E. A., Frecuş G., Rev. Roum. Biol.-Zool., 1969, **14**, *6*, 437—441.
5. Madar J., Pora E. A., Ann. Endocrinol., 1970, **31**, 1081—1086.
6. Madar J., Şildan N., Pora E. A., Arch. internat. Physiol. Bioch., 1972, 80, 367—371.
7. Madar J., Şildan N., Pora E. A., Rev. Roum. Biol.-Zool., 1973, **18**, *5*, 347—353.

8. MADAR J., ŞILDAN N., PORA E. A., Ann. Endocrinol., 1975.
9. MCLEAN P., BROWN J., GREENSLADE K., BREW K., Biochem. Biophys. Res. Comm. 1966, **23**, 117–121.
10. MUNCK A., KORITZ S. B., Biochim. Biophys. Acta, 1962, **57**, 310–317.
11. MUNCK A., Biochim. Biophys. Acta, 1962, **57**, 318–326.
12. — Perspect. in Biol. a. Med., 1971, **14**, 265–289.
13. NELSON N., J. Biol. Chem., 1944, **153**, 375–380.
14. PALMER B. G., J. Endocrinol., 1967, **37**, 351–352.

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ACTION OF STEROID HORMONES ON THE *DE NOVO* SYNTHESIS OF AMINOTRANSFERASES IN THE THYMUS OF WHITE RAT

BY

ALEXANDRU D. ABRAHAM and MARIA BORŞA

In vivo administration of a single dose of hydrocortisone stimulate RNA synthesis in the thymus of white rat after 60 min, followed by increase rate of ^{75}Se-methionine incorporation into thymic proteins. Hydrocortisone hemisuccinate stimulates especially tyrosine aminotransferase activity of thymus and liver, which effect could be abolished by simultaneous administration of mitomycin C with steroid. The effect of these steroids on the *de novo* synthesis of alanine aminotransferase and aspartate aminotransferase was studied.
Testosterone and 4-chlor-testosterone inhibits significantly tyrosine aminotransferase activity of thymus.

A number of effects of physiological levels of steroids in thymus have been shown both *in vivo* and *in vitro* including the changes in incorporation of precursors into macromolecules [1] [2] [12] [13], of RNA-nucleotidyltransferases [2] [6] [15], of transport phenomena [23] and in glucose metabolism [4] [14] [20].

In view of the considerable evidence that in many endocrine systems effects on protein and enzyme synthesis are mediated through changes in RNA synthesis and in view of the different effects of hydrocortisone on RNA-nucleotidyltransferase activity of nucleoli and extranucleolar regions of nuclei of thymocytes [21], we have extended our studies with regard to the hypothesis (a) that the stimulatory effect of hydrocortisone on chromatine RNA synthesis is an intermediary step of enzyme induction within a few hours after hormone treatment, and (b) the repression of this extranucleolar RNA synthesis and protein formation by antibiotics can suppress *de novo* synthesis of aminotransferases in thymus or not?

Furthermore, we have seen that the mechanism of action of hydrocortisone upon thymus involution differs from the mechanism of action of androgens, which fact is clear from the existence of two different macromolecular binding proteins in the thymus nuclei : 7 S-receptor for hydrocortisone and 4 S-receptor for testosterone [3] [17].

MATERIALS AND METHODS

120 male Wistar rats, weighing 100 g were used. The animals were kept under standard conditions and fasted 16 hours before treatment. Different lot of animals were injected intraperitoneally with a single dose of hydrocortisone (HC) (CIF) (3.5 mg/100 g body weight), hydrocortisone hemisuccinate (HCHS) (UMB) (5 mg/100 g), testosterone (TS) (FLUKA) (2.5 mg/100 g) or 4 chlor-testosterone (STERANABOL) (FARMITALIA) (2.5 mg 100). Other lots of animals were treated with mitomycin C (KYOWA, JAPAN) (10 μg/100 g) or mitomycin C+HCHS. The treated animals and controls were killed by exsanguinisation and thymi and livers were removed quickly and weighed on a torsion balance.

The determination of L-alanine : 2-oxoglutarate aminotransferase (AOT) (E.C.2.6.1 and of L-aspartate : 2-oxoglutarate aminotransferase (AOAT) (E.C.2.6.1.1) was carried out the method of Reitman-Frankel [16] and that of L-tyrosine : 2-oxoglutarate aminotransfer (TAT) (E.C.2.6.1.5) by Knox's method [8]. The concentration of the RNA was measured us a differential spectrophotometric method [1] and that of proteins by Lowry's technique [1 The incorporation of ^{75}Se-methionine into proteins was carried out as follows : the tissue sl were incubated at 37°C for 30 min in the presence of 0,25 μCi/ml of labeled methionine dissol in TSS-buffer [2] at pH, 7.55. After 30 min into incubation medium was added 2 ml o per cent trichloracetic acid solution. After homogenization and centrifugation (at 3000 for 20 min) the sediment obtained was washed, centrifuged and the residue was dissolved 10 per cent NaOH at 60°C 24 hours. The radioactivity of the samples were determined usi a GAMMA NC—109 type scintillation counter.

RESULTS

In vivo administration of a single dose of HC caused in the first hours after treatment significant changes of RNA concentration, of inco poration rate of methionine into thymic proteins and of AOT-, AOA and TAT activities (Figs 1 and 2).

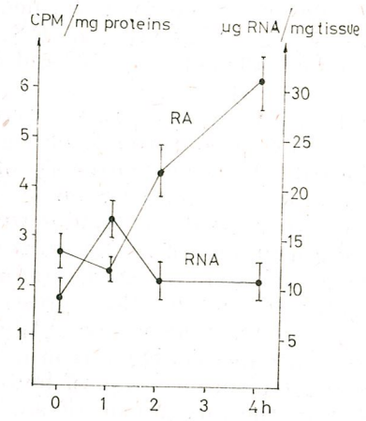

Fig. 1. — Effect of a single dose of hydrocortisone on the concentration of RNA and incorporation of ^{75}Se-methionine into proteins of thymus. (RA = radioactivity, CPM = counts/1000 × min.)

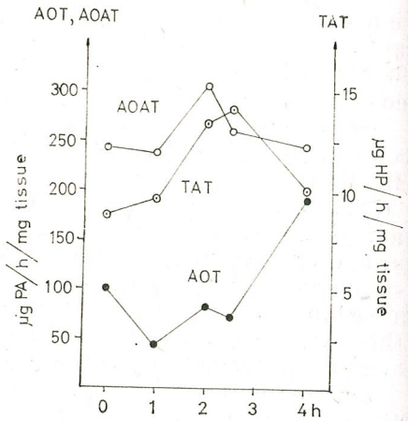

Fig. 2. — Effect of a single dose hydrocortisone on the activity of AOT, AOAT and TAT of thymus. (PA = pyruvic acid, HP = para-hydroxy-phenyl-pyruvic acid.)

In the first hour after treatment of animals with HC, significant increase of RNA concentration was observed, followed by an increase of incorporation rate of labeled methionine into thymic proteins. Slightly increase of AOAT-activity was observed after 2 hours, but the values tend to normal values after 3—4 hours. AOT-activity decreased significantly in 60 min, in 2.5 hour it increased slowly and after 4 hour the values were significantly higher against control. TAT-activity increased after 2—3 hours of treatment.

In vivo administration of a single dose of HCHS caused 20,7 per cent increase of TAT-activity of thymus after 2.5 hour, while AOT- and AOAT-activities remained unchanged. In the same time in the liver 45,9 per cent increase of TAT-activity was observed after HCHS administration (Table 1). The data concerning the effect of hydrocortisone on the liver aminotransferases are in good agreement with the observations of other authors [19].

Table 1

Effect of a single dose of HCHS on AOT—, AOAT— and TAT-activity of the thymus and liver of white rat

	AOT (μg PA/h/μg prot.)		AOAT (μg PA/h/μg prot)		TAT (μg HP/h/μg prot.)	
THYMUS						
	C	T	C	T	C	T
X	16.4	17.0	11.5	12.9	0.23	0.29
±ES	1.5	1.5	1.4	1.9	0.017	0.024
n	25	22	23	22	19	19
%	—	+ 3.6	—	+12.1	—	+20.7
P	—	> 0.05	—	> 0.05	—	>0.05
LIVER						
X	20.6	25.6	16.2	16.0	0.74	1.08
±ES	2.1	2.2	1.6	1.7	0.071	0.082
n	5	6	6	6	12	10
%	—	+24.8	—	— 1.3	—	+45.9
P	—	0.05<P<0.1	—	> 0.05	—	< 0.01

μg PA/h/μg. prot. = μg pyruvic acid per hour and μg proteins; μg=HP/h/μg prot. = μg para-hydroxy-phenyl-pyruvic acid per hour and μg proteins

X = arithmetical media, SE = standard error, n = number of experiments, % = per cent variation against control, P = Student, P.C = control, T = treated animals

The hypothesis that glucocorticoid hormones influence direcly *de novo* synthesis of aminotransferases in thymus was studied by simultaneous administration of a potent inhibitor of protein synthesis : mitomycin C with HCHS. Table 2 shows that the administration of mitomycin C inhibited evidently the stimulatory effect of HCHS on TAT-activity of thymus. Furthermore, it can be seen the existence of an inhibitory effect of mitomycin C and HCHS on AOT-activity, but no changes in the case of AOAT-activity were observed after 2.5 hour of treatment.

Administration of testosterone and of an anabolic steroid : 4-chlortestosterone, did not change AOT and AOAT-activities of thymus, but decreased TAT-activity with 41.7 per cent respectively 31,0 per cent against control (Table 3). This effect of androgen and anabolic steroids is perhaps antagonistic with that of hydrocortisone one.

Table 2

Effect of a single dose of HCHS in the absence and presence of mitomycin C on AOT–, and AOAT– and TAT-activity of thymus,

	CONTROL	MITOMYCIN C	HCHS	MITOMYCIN C + HCHS
AOT (μg PA/h/μg prot.)				
X	14.0	12.0	12.6	8.5
±SE	1.3	1.3	3.6	1.7
n	8	7	6	10
%	–	−14.3	−10.0	−39.3
P	–	> 0.05	> 0.05	< 0.05
AOAT (μg PA/h/μg prot.)				
X	8.3	8.8	9.2	10.1
±SE	2.4	3.1	3.8	2.9
n	9	8	7	11
%	–	+ 6.0	+10.8	+21.7
P	–	> 0.05	> 0.05	> 0.05
TAT (μg HP/h/μg prot.)				
X	0.16	0.16	0.20	0.15
±SE	0.012	0.013	0.013	0.023
n	6	10	10	6
%	–	0	+25.0	− 6.3
P	–	–	0.05<P<0.01	> 0.05

Table 3

Effect of a single dose of testosterone and 4-chlor-testosterone on the AOT–, AOAT– and TAT-activity of thym

	AOT (μPA/h/μg prot.)		AOAT (μg PA/h/μg prot.)		TAT (μg HP/h/μg prot.)	
	C	T	C	T	C	T
TESTOSTERONE						
X	19.8	20.3	11.3	10.6	0.36	0.21
±SE	1.9	2.0	1.8	0.9	0.06	0.04
n	8	8	8	7	8	8
%	–	+ 2.5	–	− 6.2	–	−41.7
P	–	> 0.05	–	> 0.05	–	~0.05
4-CHLOR-TESTOSTERONE						
X	16.8	14.6	8.5	10.9	0.71	0.49
±SE	2.7	2.2	2.0	1.1	0.09	0.07
n	8	10	8	10	8	10
%	–	−15.5	–	+28.2	–	−31.0
P	–	> 0.05	–	> 0.05	–	< 0.05

DISCUSSION

Very few attempts have been made to examine directly the influence f hormonal or other environmental changes on the biosynthetic rate of nzymes in high organisms. From a comparison of the relative rates of ncorporation of labeled precursors into the liver enzymes in normal and lucocorticoid-treated animals, results that the increased tissue activity erived from an increase in its rate of enzyme synthesis. These findings uggest the possibility that there is a simultaneous induction by gluco- orticoids of all the liver enzymes respective to the hormone [5] [9] [10] 22]. An increase of the rate of TAT biosynthesis already in the first hours fter treatment was observed, but in the case of AOAT synthesis this ncrease appeared only after a few days, because of the low rate of turnover f this enzyme. The half-life of TAT is 2.0 hours, while that of the AOAT .5 days [18]. The change of the biosynthetic rate of enzymes is pre- eded by an increase of RNA synthesis and of incorporation of precursor mino acids into enzyme proteins.

Slight stimulation of RNA synthesis and incorporation of amino cids into thymus proteins after glucocorticoid administration was ob- served *in vitro* in the first few minutes [24], especially the increase of extranucleolar RNA synthesis was observed, meanwhile the synthesis of rRNA is inhibited [21]. Glucocorticoid hormones produce thymocyto- lysis after 24 hours of administration [7], phenomenon which is character- zed by the considerable diminuation of DNA, RNA and protein content of thymus [20]. Histochemically could be detected some evidences of thymo- cytolysis already within this period of time, suggesting the possibility of induction of catabolic enzymes in the first few hours. Within 4 hours we observed an increase of RNA synthesis, followed by the intensification of the incorporation of ^{75}Se-methionine into thymic proteins. We believe that in this case especially mRNA synthesis is stimulated, because the RNA-nucleotidyltransferase A activity is under inhibition. The increase of TAT activity probably is due to the inductor effect of glucocorticoid hormon, which could be abolished by administration of protein synthesis inhibitor mitomycin C.

Concerning the *de novo* synthesis of AOT and AOAT, our results obtained are less convincing, because of reduced rate of synthesis of these enzymes. However, must be mentioned, that an eventually increase of enzyme synthesis in a later period of time can coincide with the disinte- gration of thymocytes, which could alterate the real interpretation of phenomena.

The mechanism of action of androgen and anabolic steroids differs from that of glucocorticoids, because testosterone and 4-chlor-testosterone inhibited TAT activity. No changes in the activity of AOT and AOAT of thymus within 4 hours after administration of these steroids was ob- served. It is known that testosterone diminuates nuclear RNA synthesis [2], suggesting partially inhibiton of mRNA containing genetic informa- tion for enzyme proteins.

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REFERENCES

1. Abraham A. D., *Teză de doctorat*, Univ. Bucureşti, 1967.
2. Abraham A. D., Sekeris C. E., Biochim. Biophiys. Acta, 1971, **247**, 562.
3. — Biochim. Biophys. Acta, 1973, **297**, 142.
4. Bartlett D., Morita Y., Munck A., Nature, 1962, **196**, 897.
5. Feigelson P., Gross P. R., Feigelson M., Biochim. Biophys. Acta, 1962, **55**, 495.
6. Fox K. E., Gabourel J. D., Feder. Proc., 1967, **26**, 424.
7. Hechter O., Halkerston D. K., Eichhorn J., *Hormonal Steroids*, Ed. Martini L., Pe A., Acad. Press. New York—London, 1964, 359.
8. Knox W. E., Brit. J. Exptl. Pathol., 1951, **32**, 462.
9. Knox W. E., Auerbach R. A., Lin C. E., Physiol. Rews., 1956, **36**, 164.
10. Knox W. E., Piras M., Tokuyama K., Enzymol. Biol. et Clin., 1966, 7, 1.
11. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., J. Biol. Chem., 1951, 1 265.
12. Makman M. H., Dvorkin B., White A. , J. Biol. Chem., 1966, **241**, 1646.
13. Morita Y., Munck A., Biochim. Biophys. Acta, 1964, **93**, 150.
14. Munck A., Perspect. Biol. Med., 1971, **14**, 265.
15. Nakagawa S., White A., Proc. Nat. Acad. Sci U.S.A., 1966, **55**, 900.
16. Reitmann-Frankel, in René Fauvert : *Technique moderne de laboratoire*, Ed. II., Mass Paris. 1961—1962, 171.
17. Schaumburg B. P., Biochim.Biophys. Acta, 1970, **214**, 520.
18. Segal H. L., Kim Y. S., Proc. Nat. Acad. Sci. U.S.A., 1963, **50**. 912.
19. Toma V., Pora E. A., Madar I., Rev. roum. biol. — zool., 1965, **10**, 39.
20. Toma V., Abraham A. D., Pora E. A., Rev. roum. biol. . . zool., 1971, **16**. 185.
21. Van Der Meulen N., Marx R., Sekeris C. E., Abraham A. D., Exp. Cell Res., 1972, 606.
22. Weber G., Bhide S. V., Mainwairing S. S., Biochem. Biophys. Res. Commun., 19 **16**, 167.
23. Young D. A., Klurfeld G., Feder. Proc., 1968, **27**, 2310.
24. Young D. A., Feder. Proc., 1970, **29**, 3006.

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LES VARIATIONS SAISONNIÈRES DE LA FONCTION THYROÏDIENNE

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The seasonal variations in the thyroid function noticed in the white Wister rats, the Brown breed cows and the rust-coloured Tzigaia breed rams can be regarded as signs of a circannual rhythm. The thyroid activity studied, throughout the year, at the level of the biochemical support of the hormonal secretion (the ribonucleic acid concentration in the gland tissue) and of the specific circulation hormones [protein-bound iodine (PBI)] point to an evolution in inverse ratio to the atmospheric temperature. It is necessary to take into account this variable when interpreting, ordering and systematizing the biological data.

Les fonctions endocrines, comme tous les processus physiologiques d'ailleurs, ne se déroulent pas avec une intensité constante. Elles présentent des variations, dans la grande majorité des cas, avec des déroulements rythmiques connectés aux différents repères temporels externes : l'alternance obscurité-lumière, la succession complète des phases de la lune, la variation saisonnière du complexe climato-météorologique, la rythmicité pluriannuelle de certains phénomènes astronomiques, etc.

L'évolution de la fonction thyroïdienne, par rapport au facteur saisonnier, a été poursuivie par de différents critériums (examen histologique, métabolisme basal, iode total et iode protéique dans le sang, radioiodocaptation thyroïdienne) chez différentes espèces d'animaux : poissons [18] [20], animaux de laboratoire [2] [11], bovidés [13—15], ovidés [7], animaux sauvages [5], et chez l'homme [9] [12] [17] [19]. On a signalé des variations saisonnières de l'iode protéique dans le lait [1] [3].

Les recherches citées par la littérature ont confronté seulement les données de deux saisons, ou bien elles ont employé des résultats partiaux, de différentes années, sans avoir une continuité absolument nécessaire dans l'interprétation des déploiements rythmiques.

MATÉRIEL ET MÉTHODE

Dans une première étape de nos recherches concernant l'évolution saisonnière de la fonction thyroïdienne, nous avons cherché à préciser, chez le rat blanc Wistar les variations annuelles, enregistrées pendant 3 années, de certains indices thyroïdiens, proportionnels à la fonction de la glande : l'iode protéique (PBI), représentant la fraction hormonale liée aux protéines sériques de transport, et l'ARN thyroïdien, le support biochimique de la sécrétion hormonale au niveau de la thyroïde.

Dans la deuxième étape nous avons enregistré les variations saisonnières de l'activité thyroïdienne, chez les vaches de race Brune, par des déterminations de l'iode protéique (PBI) dans le sang et dans le lait.

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La troisième étape des recherches a enregistré dynamiquement la réponse thyroïdie des ovinés aux oscillations spontanées de la température atmosphérique, survenues d'un j à l'autre, le facteur thermique étant probablement un des éléments principaux dans l'interp tation des biorythmes saisonniers. Dans ce but nous avons poursuivi par des dosages effect dans le même échantillon de sang, les variations de l'iode protéique (PBI) et la fixation éryth cytaire *in vitro* de la T_3-^{131}I (la preuve Hamolsky) chez les béliers de race Tzigae couleur rouille, et nous avons rapporté les données biologiques aux températures moyennes pour 24 avant les moments des investigations, échalonnées pendant la saison froide (janvier-févri

Les animaux de l'expérience ont été entretenus dans des conditions naturelles et la nourriture standardisée pour l'espèce respective, les rats blancs dans la biobase, les vac et les béliers dans les fermes.

Pour doser l'iode protéique (PBI) dans le sang et le lait on a employé la méthode de l'in nération alcaline [16]. L'extraction de l'ARN a été exécutée d'après la méthode Gie Schramm [6], modifiée par Liebenow et Schmidt [10] pour les tissus, et la concentration extraits a été estimée par la spectrophotométrie. La détermination du fixage de la T_3-^{131}I les érythrocytes *in vitro* a été exécutée d'après la technique Hamolsky [8], adaptée par Berle et collab. [4].

RÉSULTATS ET DISCUSSIONS

I. La figure 1 présente comparativement les courbes annuell moyennes des variations de l'ARN dans le tissu thyroïdien chez le r blanc, établies sur un lot de 384 animaux. On remarque un rythme à l'a

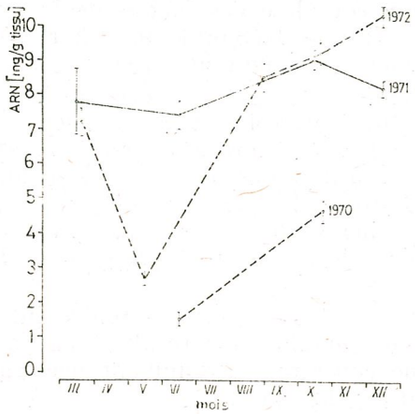

Fig. 1. — Comparaison entre les courbes annuelles des variations de l'ARN thyroïdien chez le rat (valeurs moyennes)

plitude réduite en 1971 et un rythme annuel net à grande amplitude en 197
— une diminution accentuée de l'ARN le printemps, vers un minimum e été, ensuite une augmentation progressive vers un maximum enregistr en décembre. On constate que les deux moments d'investigation de jui et d'octobre 1970 donnent une différence nettement significative, l sens de leur évolution coïncidant à celui des variations enregistrées dan la même période dans les années 1971 et 1972.

En ce qui concerne l'iode protéique dans le sang, les deux courbe annuelles enregistrées en 1971 et 1972 évoluent pareillement, en présentan des valeurs baissées dans les mois d'été et des niveaux maximums pendan l'hiver. L'image des variations annuelles de l'iode protéique déroulé sur tout l'intervalle des recherches, reflète la même conclusion (Fig. 2

La totalité des données, réunies pendant ces trois années tenant compte du critère des saisons, met en évidence, pour les deux indices thyroïdiens, des niveaux maximums pendant l'hiver et minimums pendant l'été et des valeurs intermédiaires le printemps et l'automne, les valeurs de l'automne étant légèrement supérieures à celles du printemps (Fig. 3).

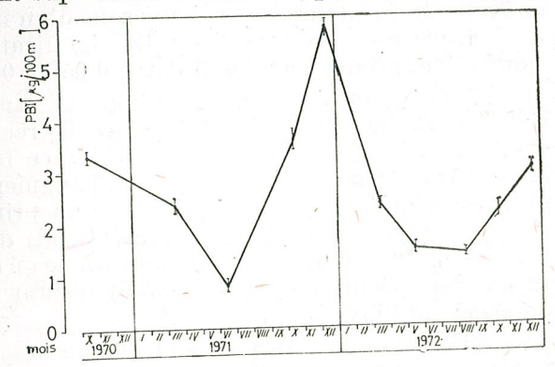

Fig. 2. — Variations du PBI chez le rat dans la période octobre 1970 — décembre 1972 (valeurs moyennes)

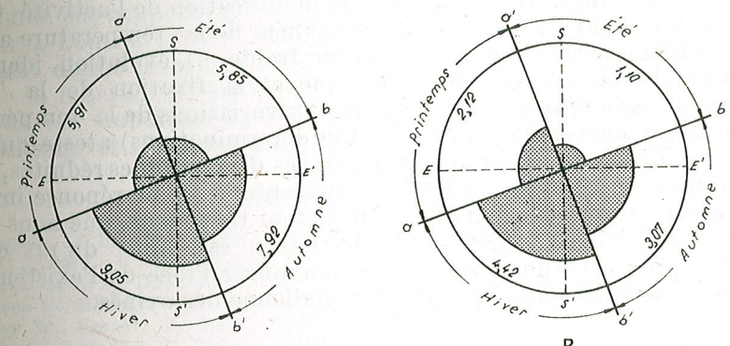

Fig. 3. — Variations de l'ARN thyroïdien (*A*) et du PBI (*B*) chez le rat, rapportées aux saisons $S-S'$ = axe des solstices ; $E-E'$ = axe des équinoxes ; $a-b$ et $a'-b'$ = axes de division usuelle des saisons. Les chiffres représentent dans l'image A : mg/g tissu humide et dans l'image *B* : µg/100 ml.

Nous considérons que la persistance des phases du rythme plaide pour l'existence d'un rythme de fond, de nature endogène prédominante, bien stabilisé et synchronisé par le photopériodisme, le facteur saisonnier strictement stable. Sur ce rythme de fond se superpose l'action des facteurs saisonniers variables, d'une annéee à l'autre et d'un jour à l'autre, parmi lesquels la température atmosphérique serait le facteur essentiel, qui modifie le niveau et l'amplitude du rythme de fond.

II. Chez les bovins les recherches ont été effectuées dans une première étape sur deux lots appartenant à la ferme « 9 Mai-Otopeni ». Le I^{er} lot contenait 20 vaches âgées de 3 à 5 ans, et le II^{e} lot 20 vaches âgées de 9 à 12 ans. Le niveau de l'iode protéique dans le sang (µg/100 ml) a été, chez le I^{er} lot de 5,5 pendant le printemps, 5,0 l'été, 5,8 l'hiver et chez le II^{e} lot de 5,0 pendant le printemps, 4,7 l'été, 5,4 l'hiver ; le niveau de l'iode pro-

téique dans le lait (μg/litre) a été, chez le I^er lot, de 59,0 pendant le printemps, 54,0 l'été, 65,0 l'hiver et chez le II^e lot de 52,0 pendant le printemps, 49,0 l'été et 59,0 l'hiver. Des données présentées il résulte que la thyroïde est plus active pendant la saison froide, les valeurs maximums de l'iode protéique étant enregistrées en hiver, tant dans le sang que dans le lait ; au cours du printemps les valeurs sont d'un niveau moyen, et elles deviennent minimums pendant l'été. La signification statistique de ces variations est exprimée par des p entre 0,05 et 0,001.

Nous avons eu la confirmation des variations saisonnières de l'activité thyroïdienne chez les bovins, par les recherches effectuées à la ferme Băneasa sur des vaches de la même race investiguées en janvier, avril et octobre. On constate qu'avec l'augmentation de la température atmosphérique, en passant de l'hiver au printemps, la concentration de l'iode protéique dans le sang (μg/100 ml) diminue significativement de 6,72 à 5,02 ($p < 0,01$) ; pendant l'automne on enregistre une légère augmentation, les valeurs (la moyenne 5,6) restant quand même sous le niveau de celles de l'hiver.

III. Dans la figure 4 sont rendus les résultats des recherches effectuées sur 10 ovinés. On a poursuivi la modification de l'activité thyroïdienne produite par les oscillations spontanées de la température atmosphérique enregistrées pendant la saison froide. L'évolution identique des indices thyroïdiens — l'iode protéique et la fixation de la T_3-^{131}I sur les hématies *in vitro* — par rapport aux variations de la température du milieu (des courbes moyennes sur 139 déterminations) ateste qu'aussi dans des conditions d'oscillations thermiques d'amplitudes réduites, enregistrées d'un jour à l'autre, la thyroïde présente la même réponse inversement proportionnelle à la variation du facteur thermique, que nous avons enregistrée au niveau du biorythme thyroïdien saisonnier du rat et des bovins. Cette observation nous permet d'avancer l'idée de l'existence du biorythme saisonnier dans l'activité thyroïdienne des ovinés.

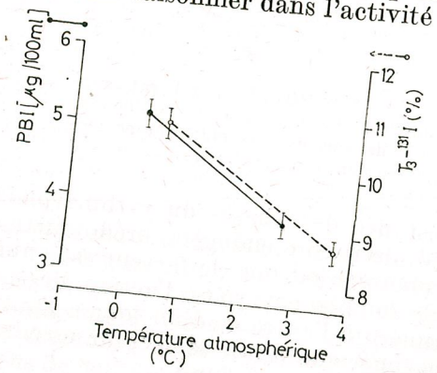

Fig. 4. — Considération comparative de l'évolution du PBI et de la T_3-^{131}I chez les ovinés par rapport aux oscillations spontanées de la température atmosphérique. Les points du graphique représentent les moyennes des valeurs du PBI et respectivement de la T_3-^{131}I dans les deux moitiés (−1 à +1,5°C et +1,5 à +4°C) dont on a divisé conventionnellement la gamme des températures enregistrées pendant ces investigations.

Dans le processus général d'adaptation aux oscillations du facteur thermique on considère le mécanisme de stimulation de la thyroïde au froid comme une réaction consécutive à la consommation intensifiée des hormones thyroïdiennes dans les tissus, la diminution de l'hormonémie par l'alimentation de la consommation périphérique déclanchant par le

feed-back des quantités augmentées de thyréotrope hypophysaire et la riposte hyperfonctionnelle de la thyroïde.

CONCLUSIONS

Les résultats de nos recherches, concernant le biorythme saisonnier de la fonction thyroïdienne, imposent les conclusions suivantes :

— Les variations saisonnières de la fonction thyroïdienne peuvent être considérées des manifestations d'un rythme circannuel, ayant une période d'approximativement une année. Au cours d'une année l'activité thyroïdienne du rat blanc, des bovins et des ovinés inscrit des niveaux maximums pendant l'hiver et minimums pendant l'été, tandis qu'au printemps et en automne les valeurs sont intermédiaires, avec une légère augmentation du niveau de l'automne.

— On peut affirmer que la relation de proportionnalité inverse entre la réponse thyroïdienne et les oscillations de la température atmosphérique se trouve non seulement pour des amples variations saisonnières, qui se traduisent dans la fonction thyroïdienne par son biorythme saisonnier, mais qu'il y a aussi une adaptation particulièrement fine de l'activité thyroïdienne par rapport aux oscillations d'amplitude réduite, enregistrées d'un jour à l'autre.

Etant donné la constance et l'amplitude des modifications saisonnières de la fonction thyroïdienne, nous soulignons la nécessité de tenir compte de cet important facteur de variabilité dans l'interprétation, la hierarchisation et l'homologation des dates biologiques.

BIBLIOGRAPHIE

1. Aizinbudas L. B., *Biologhiceskaia roli ioda*, Moskva, A.N.S.S.R., 1968, 65.
2. Aron C., Asch L., Gandar R., C.R. Soc., Biol., 1957 (1958), **151**, *11*, 1951.
3. Azurmanian E. A., Dokladi TSHA, 1966, **127**, 25.
4. Berlescu E., Bădescu M., Georgescu Gh., Toma E., St. cerc. endocr., 1964, **15**, *4*, 333.
5. Eickoff W., Frankf. Z. Path., 1957, *1*, 11.
6. Gierer A., Schramm G., Nature, 1956, **11**, 138.
7. Griffin S. A., Henneman H. A., Reineke E. P., Amer. J. Veter. Res., 1962, **23**, 109.
8. Hamolsky M. W., Golodetz A., Freedberg S., J. Clin. Endocr. Metab., 1959, **19**, *1*, 103.
9. Lewitus Z., Hasenfrantz J., Toor M., Massry S., Rabinowitch E., J. Clin. Endocr., 1964, *24*, 1084.
10. Liebenow W., Schmidt D., Acta Virologica, 1959, **3**, 168.
11. Mullick D. N., Indian J. med. Res., 1959, *4*, 393.
12. Nakano T., J. Japan Soc. Food. Nutr., 1959, **11**, 319.
13. Pipes G. W., Bauman R. T., Brooks J. R., J. Animal. Sci., 1963. **22**, *2*, 476.
14. Premachandra B. N., Pipes G. W., Turner C. W., J. Dairy Sci., 1958, **41**, *11*, 1609.
15. Seren Eraldo, Di Francia M., Atti Soc. ital. Sci. veter., 1972, **26**, 184.
16. Stolć V., *Estimation of iodocompounds in biological material*, Publishing House of the Slovak Acad. of Sci., Bratislava, 1966.
17. Suzuki S., Kawada S., Oshima S., Nagamine S., Kuga T., Yamakawa K., Japan J. Nutr., 1958, **16**, 173.
18. Swift D. R., J. Exp. Biol., 1959, **36**, *1*, 120.
19. Watanabe G. I., Uematsu M., Horii K. I., J. Clin. Endocr,. 1963, **23**, 383.
20. Woodhead A. D., J. Mar. Biol. Ass. U. Kingd., 1959, **38**, *2*, 407.

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A WORKING MODEL FOR THE SYNCHRONIZATION OF LIGHT IN PHASE SHIFTING BURBOT, *LOTA LOTA* L. (PISCES, GADIDAE) AT THE POLAR CIRCLE*

BY

ROLF KRONELD

This investigation studies the rhythmic behaviour of the burbot, *Lota lota*. The burbot, the main activity of which is concentrated to the morning and the evening, tends to move its main point of activity into the day during the autumn, when the length of the day on northern latitudes is extremely shortened. This behaviour is called phase shift and seems to be ruled by a determined light optimum. The light conditions seem to synchronize several underlying endogenous master-reactions.

Thus, the rhythmic behaviour of the organism does not necessarily have to be ruled by any oscillator system, a hypothesis that has been much discussed. The behaviour of the organism seems most nearly to be a reaction following variations of light under certain light optima.

The theoretical working hypotheses for rhythmic behaviour that have been presented in literature up to now are built on one or several oscilllating systems [1] [2] [5—7] [11] [13] [14] [17]. Thus, Pittendrigh and Hoffmann suggest that the rhythm of activity in vertebrates is controlled by at least two oscillators. Gwinner proposes a two-oscillatosystem with one light-active and one dark-active oscillator [2]. Müller, Andreasson, Eriksson and Erkinaro also discuss the possibility of an existing light-resp. darkness-oscillator. This would make it possible for the organism to react either as day-active or as night-active. The phase shift would then neutralize the influence of external, extreme Zeitgeber-factors.

Hoffmann also considers the possibility that the two oscillators might have two stable relationships in relation to each other. They can either be in phase and cooperate, or out of phase with a difference of 180°, which creates a double pattern of activity. Andreasson believes that the extreme light conditions in certain occasions would make impossible a synchronixation of these two oscillators in a uniform day-activity pattern. Hoffmann has also shown that a drop in light intensity below a certain threshold initiates a splitting of the two oscillators he has hypothetized.

The results of the forthcoming piece of research can be interpreted as further proof of a two- or multioscillator system in burbot. Nevertheless it is also possible to regard phase shift as a pattern of different ac-

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tivities depending of a relative optimum of illumination and independent of for instance two different activity systems.

It can be presupposed that this optimum of illumination might give rise both to phase shift and splitting phenomena.

This optimum is probably a relative value based on summation of the light intensity and day length of several days [6].

These results, which are based on the behaviour of the burbot in various light conditions in laboratory surroundings, should not be applied to burbots in nature without further control.

MATERIAL AND METHODS

This piece of research is based on observations of a group of burbots consisting of fou individuals in age class 0+ (5—7 cm). The investigation was made in Messaure (66°42′N, 20°25′E), 20 km north of the Polar Circle. The fishes were caught in the Kaltisjokk River and had possibilities to adapt to the experimental vehicles before the beginning of the investigation. The fishes were being fed with tubificides and the feeding occurred with irregular intervals during the activity period of the fish in order to avoid influencing of the activity pattern.

The fish phase shifted to day-activity in the beginning of October [8] [15], at the onset of the experiments, whereafter the freezing of the waters, which occurs in nature, was imitated by covering the aquaria with sheets, leading to a lowering of light intensity [12].

The light conditions, which during the phase shift changes from 10^3 — 10^2 lux, decreaser due to the moderation to 10^2—10^{-1} lux. The change in light intensity which precipitates the phase shift is thus approximately the same degree as the change (the freezing of the waters) which induces splitting of the activity.

The locomotory activity has been registered and the number of passages has been counted in one-hour intervals on an Elmeg counter. The registration was done with redlight- photocell according to Müller and Schreiber [16]. The number of passages has been described as absolute values of ten-days periods.

RESULTS

A group of burbots in the age class 0+ (four individuals, see figure 1*A*), which already have phase shifted in natural light conditions (nLD) earlier during the autumn, were exposed to a light intensity moderation experiment. After the light moderation the clear dominance of daytime activity disappears, and in december (decades II — III) the fish is almost night-active. This occurs at a time when the burbot as a rule shows a day-active behaviour [10] [12]. In fig. 1B it is shown how night-active burbots phase shift to day-activity according to the light moderation and the rule of Müller [12]. The light conditions during the moderation are presented in figure 2, which shows the values for a number of representative days during the experiments.

DISCUSSION

The number of activity peaks that can be shown in these experiments does not exceed a number of six.

At a complete synchronization, either when the fish is day-active or night-active, the number of activity peaks is = 1. At decreasing

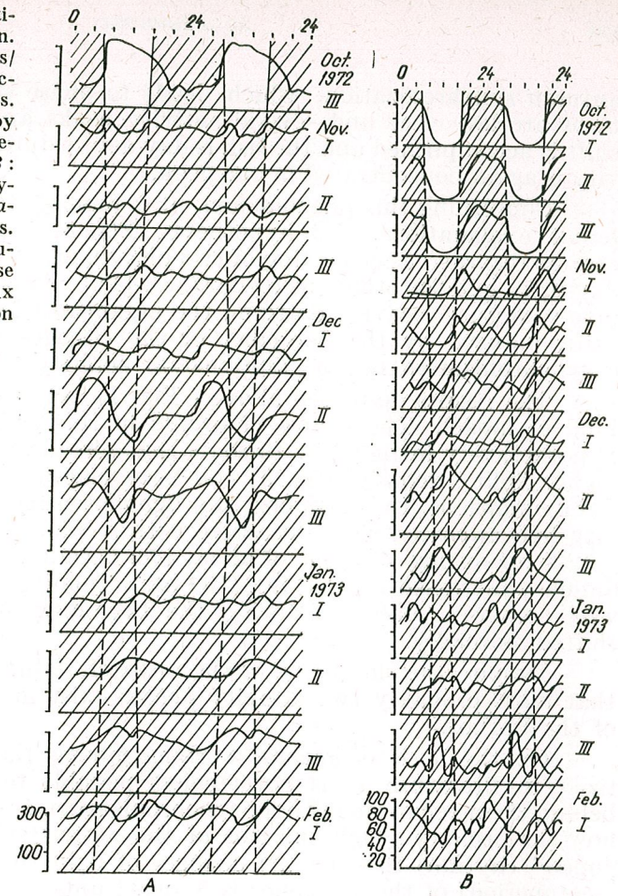

Fig. 1. — *A* : Splitting of activity through light moderation. *Ordinate* : number of passages/2 hours. *Abscissa* : average activity during monthly decades. Sunrise and sunset marked by 5-lux limit values. Light moderation marked by lining. *B* : Phase shift from night- to day-activity in the burbot. *Ordinate* : number of passages/2 hours. *Abscissa* : average activity during monthly decades. Sunrise and sunset marked by 5-lux limit values. Light moderation marked by lining.

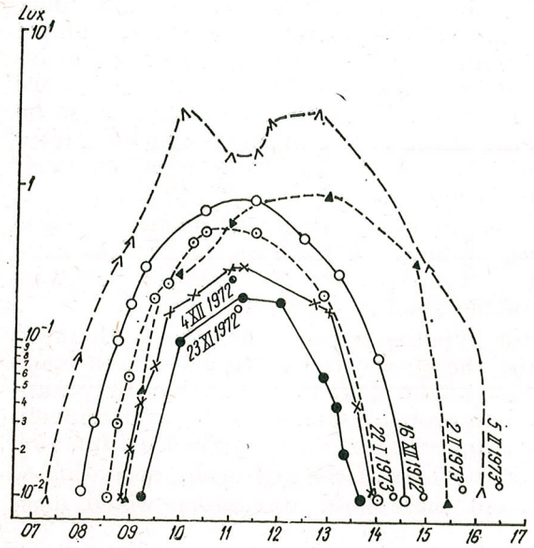

Fig. 2. — Light values for a number of representative days during the light moderation (O = clear, ◐ = partly cloudy). *Ordinate* : light intensity in lux (logaritmically). *Abscissa* : time of the day (hours).

autumn synchronization, which leads to phase shift, the activity patterns are less clear and several activity peaks appear. When the phase shift is accomplished and the fish is day-active during the winter, the activity is again concentrated in one peak.

Concerning this phenomenon, it is possible to consider several different explanations, and hypotheses. Fig. 3, explains how the activity can be regarded as consisting of two phases, of which one is influenced by day-light and the other one by darkness [14]. By cooperation these phases give a unitary activity pattern; the organism is synchronized. At phase shift, induced by the Zeitgeber or the changes in light and darkness, desynchronization occurs, and several activity peaks appear.

The results described above (Fig. 1) can be seen according to this kind of model. The course of events in this case have been described hypothetically in figure 4. Burbots that already have phase shifted at a higher light intensity (Fig. 3) had been placed in extremely diminished light conditions. In December, at a light intensity of ca. 0.1 lux, the activity had appeared as night-active with a "double-pattern" of rhythmic behaviour during a period of about two decades. When light intensity in January increased to 2—3 lux (10^1 lux), "joining" patterns of behaviour were observed. Later on during the beginning of spring the fish phase shifted normally.

Despite the simplicity of the research design, the results indicate that most probably two activity phases exist in the rhytmic behaviour of the burbot.

On the basis of general working models that have been hypothetized in literature it is nevertheless possible to outline the principles behind the mechanisms of light synchronization. In figure 5, it is explained how the primary synchronizator of the activity rhythm (the biologically "open" rhythm) through the changes of light (fotoperiod) and darkness (scotoperiod) of the Zeitgeber is induced unto consistence with the Zeitgeber. This synchronizator influences and is influenced by the inner secondary synchronizator of various bodily rhythms through for example "feed-back" mechanisms of different kinds. The endogenous rhythm includes species-specific patterns of behaviour and function in consistence with the open biological rhythm and the Zeitgeber. Generally, it can be said that the Zeitgeber through the effect on the primary synchronizator establishes a definite and adaptive phase relationship between the inner rhythm and the surrounding environment.

The synchronizators are probably of an integrated nature. Because of this, operative incisions with removal of light receptors that have been made up to now have not been able to give any definitive answers as regarding to the localization of the "biological clock" [4] [9].

As considering the oscillator hypotheses concerning the rhythmicity of the burbot, one has to regard the observations that have been made of several peaks of activity. These might make it reasonable to presuppose a multioscillator-system which probably underlies the by Hoffmann presumed two oscillators. These two oscillators again might then function as a synchronizator for the inner rhythms of the organism, according to figure 5. In Pittendrigh's system these both oscillators would most

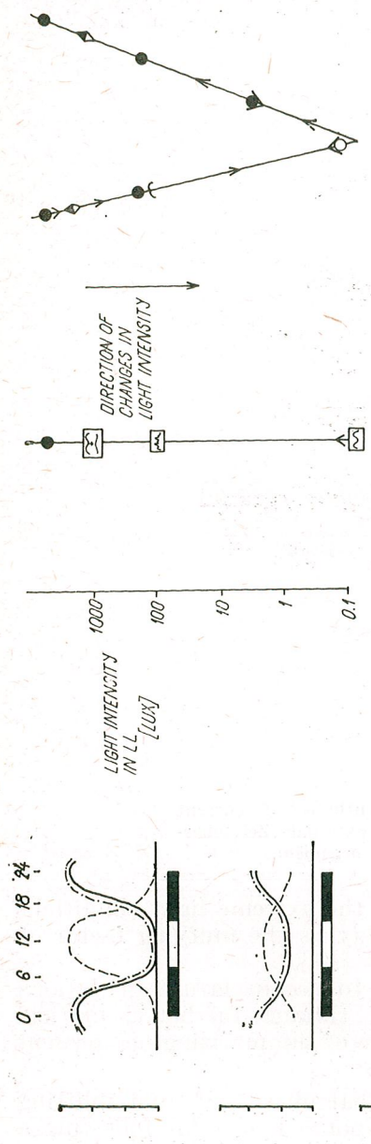

Fig. 4. — Hypothetical working model for splitting of activity in the burbot as a function of light-intensity lowering. A, describes the course of events according to figure 1, and B, describes the total course of the fish-activity.

Fig. 3. — Hypothetical working model showing activity as consisting of two phases, of which one is seen as influenced by day-light and the other by darkness. Double lines = biological open rhythm of activity (registered rhythm). Single lines : underlying, light — or alternatively darkness- steered rhythm (hypothetical rhythm). Both rhythms are inclined in the so-called primary synchronizator.

nearly correspond to the ESSO A system. The synchronizator of the inner rhythms would correspond to the ESSO B system.

The underlying peaks of activity which were earlier mentioned describe the inner rhythms and would, seen from a physiological-biochemical point of view, signify definite steering "master-reactions".

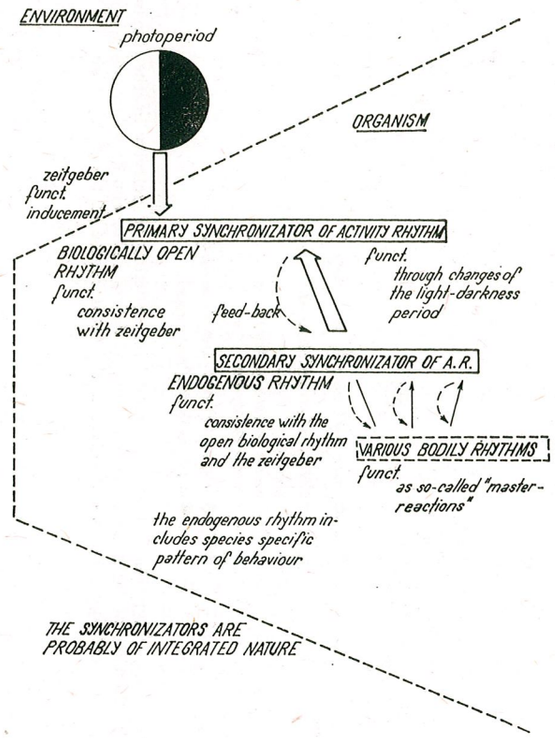

Fig. 5. — Hypothetical model and synthesis of current knowledge of cooperation between external Zeitgeber-rhytms and the rhythm of the organism.

However, it is also possible to regard the extreme light conditions on northern latitudes with the shortening day as the unifying factor of the morning and the evening activity peaks [18].

Despite gross variances as regarding to nomenclature in various authors, the outlines that been given might, through further theoretical and experimental analyses, serve the purpose of summarizing our present knowledge of biological rhythms.

As regarding figure 4, the possibility that phase shift and splitting of activity might be a direct pattern of response behaviour following a decrease in light intensity under a certain light optimum. Such a pattern of response behaviour does *not necessarily* have to be a result of an underlying oscillator system, even if experimentally two phases of activity can be distinguished.

This light optimum, or the light intensity that precipitates these patterns of behaviour, ought to be more closely determined. At the present moment this type of research encounters the following difficulties :
1) the laboratory conditions diverge from nature in the respect that the reciprocal relationship between individuals and the nature does not occur in the studying of separate individuals.
2) sufficient exacticity in determining the light optimum mentioned above is lacking ; this should be investigated by means of a sufficient number of parallell trials.

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LITERATURE

1. Andreasson S., Day-och nattaktiva simpor. F.o.Fl, 1972, **67**, 229—232.
2. Aschoff J., *Circadian rhythms in birds.* Proc. 14th Int. Ornithol. Congr., 1967, 81—105.
3. Eriksson L., Ber. Ökol. Stat. Messaure, 1971, *8*, 1—29.
4. — Aquilo Ser. Zool., 1972, **13**, 8—12.
5. — Ber. Ökol. Stat. Messaure, 1973, **23**, 33—40.
6. Erkinaro E., Fasväxling hos djur. F.o.Fl., 1972, **67**, 215—219.
7. Hoffmann K., Biochronometry ISBN 0—309—01866—8, 1971.
8. Kroneld R., *Diel-and seasonal rhythmicity in different age classes in the burbot Lota lota L. at the artic circle,* Oulanka rytmikkonf., NKTE publ., 1972.
9. — *Diel rhythmicity in the locomotory behaviour of eye-eliminated burbot Lota lota L. (Pisces, Gadidae) at the polar circle during the winter,* 1974.
10. Müller K., Umschau 69, 1969, 18—19.
11. — Oikos Suppl. 13, 1970, 108—121.
12. — Oikos Suppl. 13, 1970, 122—129.
13. — F.o.Fl., 1972, **67**, 23—29.
14. — J. Comp. Phy., 1973, **84**, *4*, 357—361.
15. — Ber. Ökol. Stat. Messaure, 1973, **23**, 9—20.
16. Müller K., Schreiber H., Oikos, 1967, **18**, 135—136.
17. Pittendrigh C. S., *Life Sciences and Space Research* Proc. on Int. Space. Sci. Symp. (Wien 1966) North-Holl., Amsterdam, 1967, 122—134.
18. Wikgren Bo—J., Mem. Soc. F. Fl. Fennica, 1956, **31**, 91—97.

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