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SOMMAIRE

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UN NOUVEAU GENRE ET UNE NOUVELLE ESPÈCE DE LA FAMILLE *MYCOBATIDAE* (ORIBATEI)

PAR

Z. FEIDER, N. VASILIU et MAGDA CĂLUGĂR

Le nouveau genre *Allozetes* n.g. est caractérisé par la forme de la translamelle et le nombre de poils sur les volets génitaux. La nouvelle espèce *Minunthozetes tarmani* n.sp. présente un sensillus de forme particulière.

Parmi les Oribatides de la famille *Mycobatidae* Grandjean 1954 on a cité en Roumanie *Mycobates tridactilus* Willmann, 1929.

Dans ce travail, parmi les espèces de la famille mentionnée, nous décrivons un genre nouveau : *Allozetes* n.g., d'après l'espèce type *Minunthozetes latus* Schweitzer, 1956, et une nouvelle espèce pour la science : *Minunthozetes tarmani* n.sp. (dédiée à l'acarinologue K. Tarman) [1], [2], [3], [4], [5], [6], [7], [8], [9], [10].

Genre **A l l o z e t e s** n.g.

Le genre *Allozetes* n.g. diffère du genre *Minunthozetes* duquel il a été isolé par plusieurs caractères distincts : 1. Les ptéromorphes ne dépassent pas antérieurement le notogaster. Vue latéralement, la ptéromorphe est attachée dans toute sa longueur, à savoir non seulement dans sa partie antérieure, au niveau du pont de la ptéromorphe. 2. La translamelle est réduite à un épaissement linéaire et n'est pas évidente comme chez le genre de comparaison. 3. La lyrifissure dorsale est disposée médialement par rapport à la ligne qui réunit les deux aires poreuses. 4. Le poil V est fixé entre les lignes longitudinales qui passent par les poils VI

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et VII. 5. Les deux poils VIII d'une partie sont fixés en avant et en arrière de la seconde aire poreuse. 6. Les volets génitaux sont munis de cinq paires de poils. 7. Les deux paires de poils XI, adanaux, sont fixés sur l'anneau anal. 8. Les quatre poils intercoxaux antérieurs sont disposés en deux rangées. 9. La coxe III présente un seul poil.

Espèce type du genre : **Minunthozetes latus** Schweitzer, 1956.

Allozetes latus (Schweitzer, 1956)

L'idiosoma est long de 241 μ et large de 174 μ. Le notogaster est ovale, tronqué antérieurement.

Le prodorsum, avec le rostre aigu, présente des lamelles proéminentes rétrécies en avant, avec les poils lamellaires dépassant le rostre. La translamelle est réduite à une simple nervure convexe en avant. Les poils interlamellaires, minces, avec la base recouverte par le notogaster, mesurent la moitié de la longueur du prodorsum. Les poils rostraux, aussi longs que les poils lamellaires, sont fixés sur un épaississement latéral. Les poils trichobothriaux larges, fusiformes, munis d'un mucron filiforme à l'extrémité distale, présentent un pédicelle assez long.

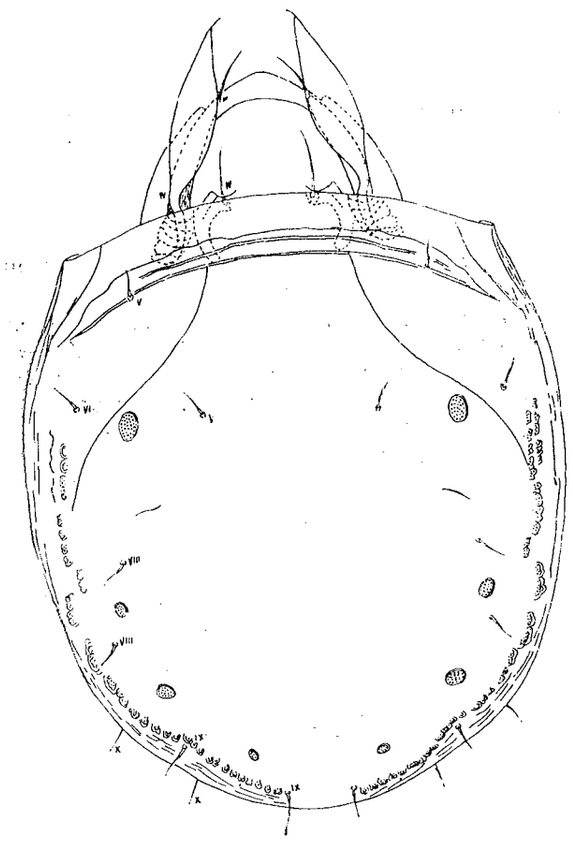

Fig. 1. — *Allozetes latus*, vue dorsale

Le notogaster, arrondi en arrière, présente les impressions musculaires, qui commencent au niveau de la première aire poreuse et s'enfilent un peu irrégulièrement (fig. 1). Parmi les quatre aires poreuses la première est la plus grande, la dernière est la plus petite, et les deux autres sont égales. Les poils sont relativement petits.

Vue latéralement, la ptéromorphe présente l'extrémité distale tronquée. Les poils de la dixième rangée sont très déplacés postérieurement (fig. 2).

Sur la face ventrale l'orifice génital se trouve un peu en avant du milieu de l'idiosoma. Les crêtes circumpédales dépassent les coxes IV.

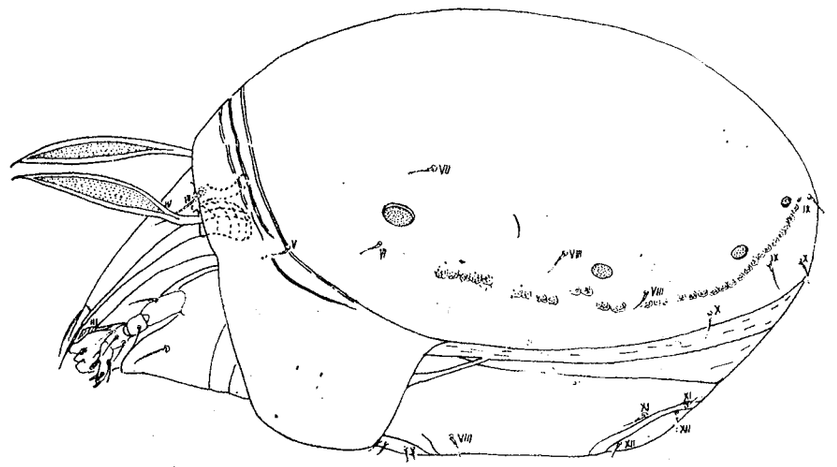

Fig. 2. — *Allozetes latus*, vue latérale

La tectopédie I est plus large dans sa moitié postérieure. Les apodèmes sont rapprochés à la ligne médiane. Les poils coxaux et intercoxaux sont dirigés en avant. L'anneau génital large présente des prolongements latéraux. Les volets génitaux ont les bords arrondis et les poils dirigés en avant. Les poils agénitaux courts et distancés sont dirigés en avant. L'anneau anal, élargi, en avant, présente, à côté des lyrifissures, les deux paires de poils XI. Les volets anaux, trapézoïdaux, ont les poils dirigés en avant. L'organe préanal est arrondi en arrière (fig. 3) [4].

Localité : Monts Parîng, localité Peştera Boli (dép. Hunedoara), feuilles mortes de hêtre, date : 27.VII.1967, un spécimen.

Minunthozetes tarmani n.sp.

L'idiosoma est long de 280 μ et large de 180 μ. Le notogaster, ovale, est tronqué à la partie antérieure et légèrement aiguisé à la partie postérieure.

Le prodorsum, avec le rostre relativement aigu et avec la partie postérieure recouverte par le pont des ptéromorphes, présente des lamelles évidentes et la translamelle aussi bien développée. Sur le bord du prodorsum on observe un épaississement qui est proéminent antérieurement et sur lequel sont fixés les poils rostraux plus courts que le rostre. Les poils lamellaires dépassent le rostre, tandis que les poils interlamellaires,

courts, ont la partie basale recouverte d'une large proéminence médiane du pont des ptéromorphes. Les poils sensilligères allongés, avec l'extrémité distale prolongée par un court filament, sont fixés dans une bothridie conique courbée. Leur surface présente une structure alvéolaire.

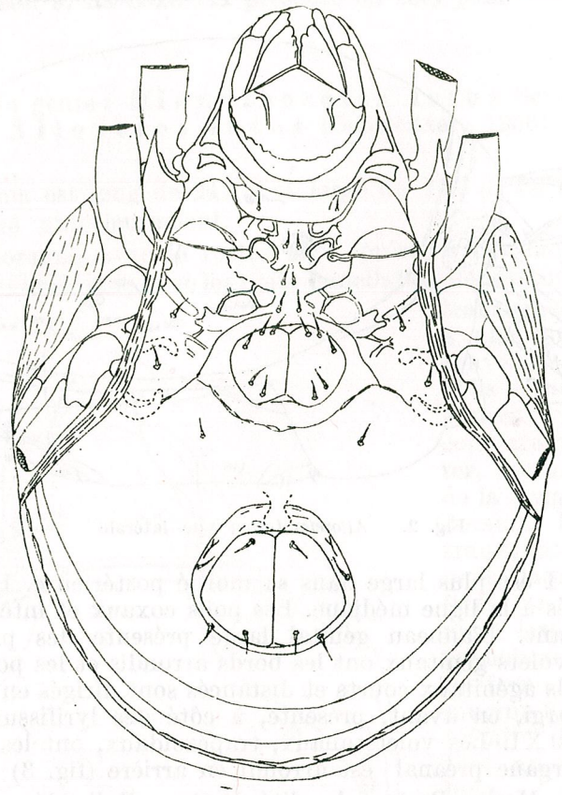

Fig. 3. — *Allozetes latus*, vue ventrale

Le notogaster, plus large vers son milieu et plus étroit au niveau des ptéromorphes, présente l'extrémité antérieure en forme d'angle. Les impressions musculaires, nombreuses et petites, sont disposées en forme de demi-cercle en commençant du niveau des lyrifissures. Les aires poreuses décroissent progressivement, et la dernière paire a les aires très rapprochées. Les poils, avec le socle bien développé, sont assez courts. La lyrifissure fine est légèrement courbée. Les deux poils VIII d'une paire sont fixés en avant de la seconde aire poreuse (fig. 4).

Vue latéralement la ptéromorphe, avec une extrémité symétriquement arrondie ne dépasse pas le bord antérieur de l'idiosoma. La dernière rangée de poils X n'arrive pas jusqu'au bord du corps (fig. 5).

Sur la face ventrale l'orifice génital est placé au centre de l'idiosoma. Les crêtes circumpédales sont étroites et dépassent à peine la coxe

IV. La tectopédie I présente des extrémités inégales et aiguës. Les apodèmes sont éloignées de la ligne médiane. Les poils coxaux et intercoxaux, avec le socle bien développé, sont dirigés en avant. L'anneau génital est arrondi en arrière. Les deux volets génitaux ont une forme pentago-

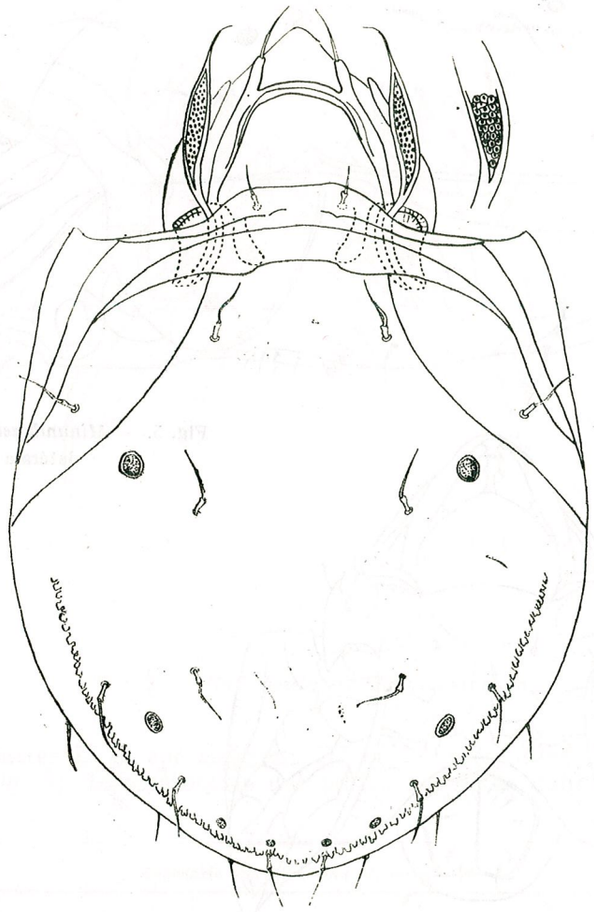

Fig. 4. — *Minunthozetes tarmani*, vue dorsale

nale et sont recouverts de six paires de poils, dont cinq sont concentriques. Les poils agénitaux sont courts et dirigés en avant. L'anneau anal plus large en avant et les deux volets, conjointement, ont une forme pentagonale. L'organe préanal est double. Les lyrifissures de l'anneau sont droites. La première paire de poils anaux et les deux premières paires de poils adanaux sont dirigés en avant. Les autres poils ont une disposition latérale (fig. 6).

Le gnathosoma présente le mentum circulaire et aigu. Sa partie antérieure relativement très développée, recouvre presque en totalité

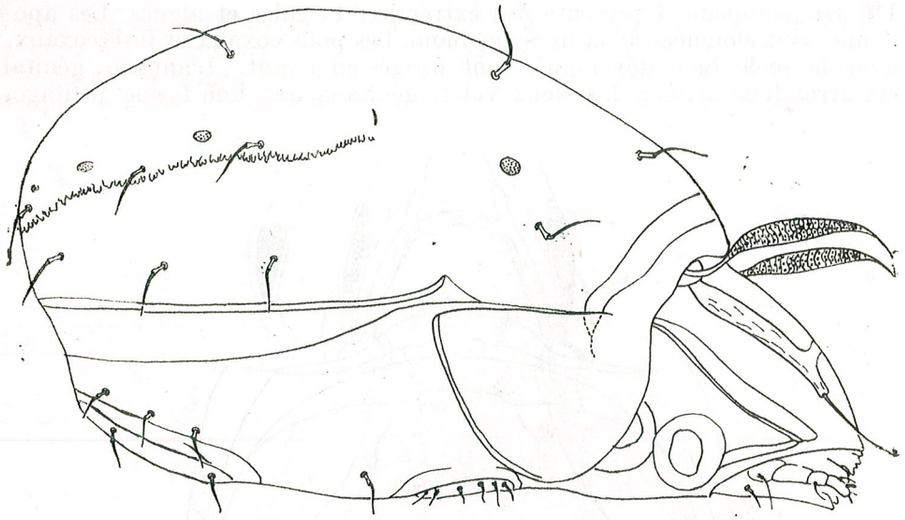

Fig. 5. — *Minunthozetes tarmani*, vue latérale

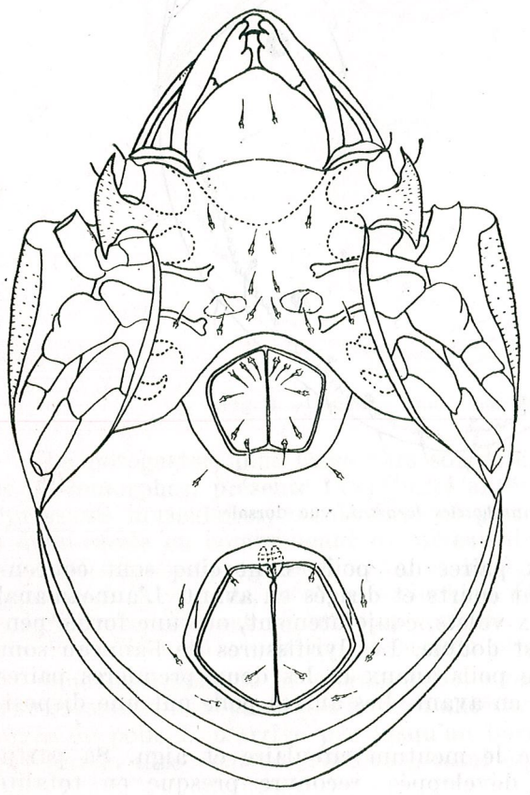

Fig. 6. — *Minunthozetes tarmani*, vue ventrale

les gènes et les poils correspondants. Les poils latéro-coxaux sont très fins. Le palpe présente deux poils fins sur le fémur, un poil sur le génual, trois poils dont un barbulé, sur le tibia, neuf poils dont un solénidion et trois eupatidies terminales sur les tarses (fig. 7).

Fig. 7. — *Minunthozetes tarmani*, gnathosoma

Les pattes II et spécialement III et IV présentent des crêtes sur le fémur (fig. 8). La chêtotaxie des pattes est notée dans le tableau 1.

Tableau 1

Chêtotaxie des pattes chez *M. tarmani*

Pattes / Article	I	II	III	IV
Trochanter	v	—	—	v
Fémur	v′, v″, d	v′, v″, l, d^1, d^2	v, d	v, d
Génual	σ, v′, v″, l	σ, v, d	σ, l	d
Tibia	φ_1, φ_2, v, l	φ, v, l, d	φ, v′, v″	φ, v
Tarse	ω_1, ω_2, (ft), (pl) (tc,) (it), (p), (u), (a), s, (pv), v A	ω_1, ω_2, ft, (tc), (it), (p), (u), (a), (pv), s, (pl)	ω, (ft) , (tc), (u), (a), s, (pv)	ω, (tc), (it), (u), (a), s, (pv)

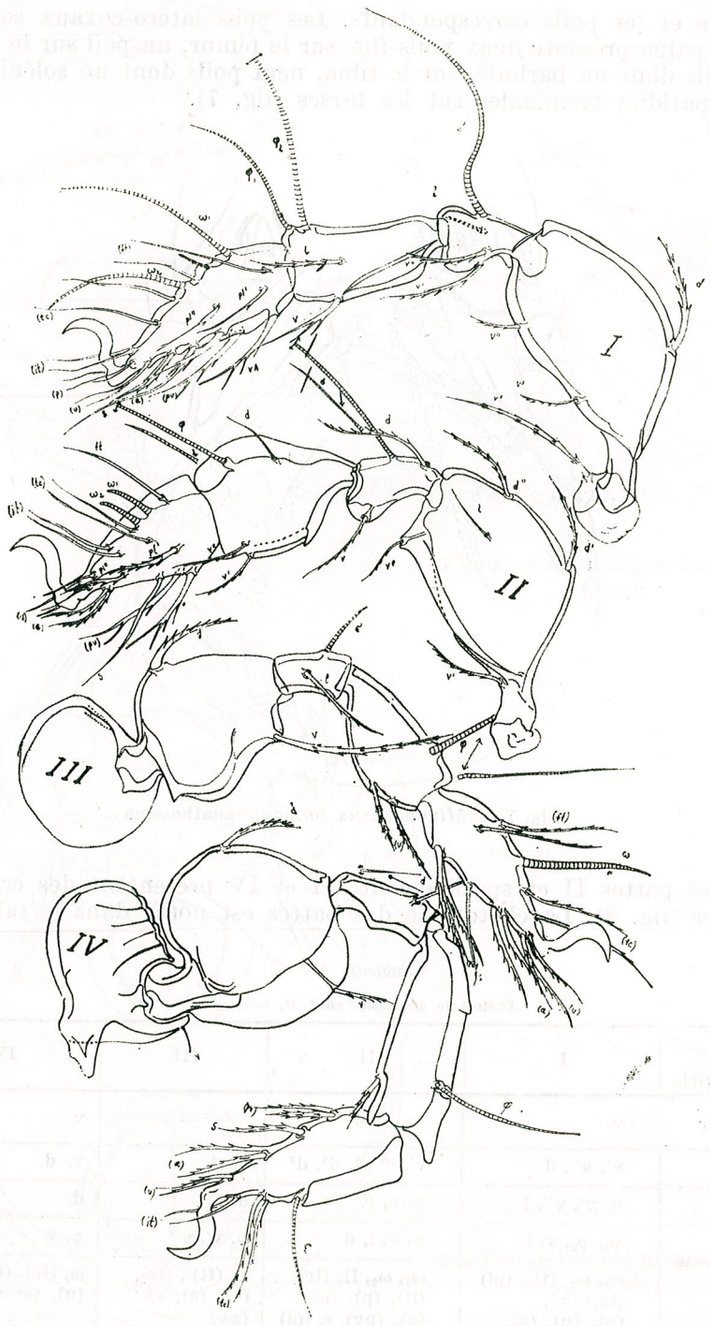

Fig. 8. — *Minunthozetes tarmani*, pattes

Holotype femelle et quatre paratypes chez les auteurs.

Terra typica : Monts Parîng, localité Peştera Boli (Hunedoara), feuilles mortes de hêtre. Date : 27.VI.1967.

Autres localités : Slătioara (Suceava), feuilles mortes de hêtre, 21.IV.1968, 10 spécimens ; Moţca (Jassy), feuilles de hêtres 15.IV.1970, 100 spécimens ; Agapia (Neamţ), feuilles mortes de conifères et bois pourri, 16.IV.1970, 45 spécimens.

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Centre de Recherches Biologiques Jassy

SUR LA FORMATION DES GENRES CHEZ LES LÉPIDOPTÈRES

PAR

EUGEN V. NICULESCU

In this paper the author puts forward a hypothesis concerning the formation of genera. The author agrees that the phenomenon is comparable with speciation, with the only difference that the restructuration of the genital armature by mutation is deeper than in speciation and results in a new structure plan, differing from the old one, while in speciation the phenomenon is occurring within the same structure plan. It is shown that genera *Heodes*, *Thersamonia* and *Palaeochrysophanus* cannot be accepted as valid genera as their structure plan does not differ from that of the genus *Lycaena*. The same thing holds for genera *Meleageria* and *Agrodiaetus*, whose species must be included in the genus *Polyommatus*.

Le problème du genre est tout aussi intéressant que le problème de l'espèce. Cette dernière a fait l'objet d'un grand nombre de travaux dont certains forment des volumes impressionnants. Rien de semblable en ce qui concerne le genre qui, par comparaison à l'espèce, a été le plus souvent ignoré sous cet aspect — quoique les systématiciens travaillent non seulement sur des espèces mais aussi sur des genres.

Comme chez les espèces, pour lesquelles se pose le problème de leur séparation précise et la question : « s'agit-il d'une espèce ou d'une sous-espèce ? » — de même chez les genres se pose aussi le problème de leur délimitation précise et très souvent il faut trancher la question : « s'agit-il d'un genre ou d'un sous-genre ? ». Tous les systématiciens ont à résoudre les questions suivantes : Quand semble-t-il nécessaire de créer un nouveau genre ? Quand sommes-nous en présence d'un genre et quand s'agit-il d'un sous-genre ? Pourquoi l'espèce X appartient-elle au genre Y et non pas au genre Z ? On voit que les systématiciens débattant de tels problèmes se trouvent souvent dans l'embarras en ce qui concerne l'octroi de rang générique ou sous-générique à un certain taxon. Le pro-

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blème étant intéressant et d'actualité, il doit être débattu dans nos revues de biologie. C'est pourquoi nous avons décidé de publier le présent article.

En ce qui concerne la délimitation des genres nous avons publié plusieurs travaux [6], [7], [8], [9], ce qui nous dispense d'y revenir. Dans ce travail nous allons essayer d'élucider le problème de la formation des genres.

Tout systématicien sait que les caractères morphologiques utilisables en taxonomie sont extrêmement variés pour un même taxon — surtout pour l'espèce et le genre. Mais ces caractères si variés sont de valeurs diagnostiques très inégales. Certains organes fournissent des caractères excellents au niveau spécifique ou générique, tandis que d'autres présentent des caractères si imprécis et fluctuants que l'établissement d'un certain taxon par l'utilisation de ceux-ci présente une grande incertitude.

Que devons-nous faire quand nous sommes en présence d'un ensemble de caractères? Il est de toute évidence que dans ce cas s'impose impérieusement leur « triage » par une judicieuse discrimination sur la base du principe de la subordination des caractères, car il est bien connu que tous les caractères n'ont pas la même valeur. Il est à peine besoin de démontrer que le « dernier mot » dans la diagnose appartient à l'armure génitale qui a le privilège d'être plus constante que tous les autres caractères et, partant, la plus précieuse.

Il arrive parfois que tous les caractères de l'« ensemble » ne concordent pas pour une même conclusion taxonomique. Il y a des cas où l'habitus et les genitalia conduisent à une certaine conclusion, mais celle-ci n'est pas confirmée par les premiers états.

Ainsi par exemple sept espèces de *Lycaena* (s.l.) de Roumanie à savoir : *Lycaena virgaureae* L., *L. tityrus* Poda, *L. alciphron* Rott., *L. helle* Den. et Schiff., *L. phlaeas* L., *L. thersamon* Esp. et *L. hippothoë* L. ont l'œuf, la chenille, la chrysalide, l'habitus de l'imago et les genitalia du type *Lycaena*, mais la huitième espèce, *Lycaena dispar* Haw. a seulement l'habitus et les genitalia du type *Lycaena*, tandis que l'œuf, la chenille et la chrysalide sont d'un tout autre type de structure. En jugeant d'après les premiers états, *Lycaena dispar* Haw. devrait appartenir à un autre genre, distinct de *Lycaena*, mais à ceci s'oppose l'armure génitale. Comment devons-nous procéder dans ce cas? Il y a deux alternatives : soit accorder la prééminence aux premiers états et ériger un nouveau genre (ou accepter le genre *Thersamonia* Verity), soit laisser l'espèce *Lycaena dispar* Haw. dans le genre *Lycaena*, en se basant sur l'armure génitale. Puisqu'il est notoire que les caractères des œufs, des chenilles et des chrysalides sont beaucoup plus variables que ceux fournis par les genitalia, nous devons donc accepter la deuxième alternative et inclure l'espèce *dispar* Haw. dans le genre *Lycaena*. Un nouveau genre, pas plus que le genre *Thersamonia* Verity, ne serait valable puisque :

1. Il ne se séparerait pas de l'ancien genre (*Lycaena*) par une discontinuité prégnante (E. V. Niculescu, 1970).

2. Il ne serait pas l'équivalent morphologique et taxonomique de l'ancien genre (*le principe de l'équivalence des caractères*, E. V. Niculescu, 1968).

3. Il n'aurait pas dans l'armure génitale un plan de structure différent de celui de l'ancien genre (*le principe de l'unité du plan de structure*, E. V. Niculescu, 1970).

Toutefois *Lycaena dispar* Haw. est plus distincte des sept autres espèces, que celles-ci ne diffèrent entre elles ; *L. dispar* Haw. a dans l'œuf, la chenille et la chrysalide un type de structure différent de celui des autres espèces du genre. Comment s'expliquer cette déviation des premiers états, l'imago restant de type *Lycaena*? S'agirait-il ici d'un phénomène anormal, un « ludus naturae », ou au contraire s'agit-il d'un phénomène biologique habituel?

En observant de plus près ces faits nous avons essayé de leur donner une explication et de les placer dans le cadre du phénomène général de l'évolution. Nous estimons qu'il s'agit dans ce cas du *primum movens* d'un nouveau genre. On sait que les genres actuels n'ont pas existé dès le début de l'évolution. Il a existé une période pendant laquelle les Lépidoptères étaient représentés par d'autres genres que ceux existant actuellement et, à une époque plus reculée, il n'y avait aucun Lépidoptère mais seulement un groupe lépidoptéromorphe primitif — l'ancêtre de l'ordre actuel des *Lepidoptera*. Les genres actuels sont apparus au fur et à mesure, en se transformant les uns dans les autres, de même que les espèces qui se transforment, elles aussi, les unes dans les autres. Le processus évolutif qui aboutit à l'apparition d'un nouveau genre ressemble fort à celui de la spéciation. Le nouveau genre n'apparaît pas d'emblée par un phénomène mystérieux. Comme la spéciation est précédée d'un long phénomène de sous-spéciation (E. V. Niculescu, 1961), l'apparition d'un nouveau genre est, de même, longuement préparée par un phénomène d'accumulation des différentes modifications quantitatives — morphologiques et écologiques — sous l'influence des mêmes facteurs du milieu externe. Aujourd'hui nous rencontrons des « genres en cours de transformation » aux divers stades de cette dernière.

A ce qu'il paraît le premier stade est celui de la modification de l'habitus. Celui-ci est plus influençable par les facteurs du milieu externe — la preuve en est la variation individuelle si large chez la plupart des espèces. Nous en trouvons justement un bon exemple dans le genre *Lycaena*. Ainsi chez *Lycaena virgaureae* L. nous constatons une tendance à la modification de l'habitus. Si le dessus des ailes est nettement de type *Lycaena*, le dessous des ailes postérieures présente un type de dessin différent de celui des autres espèces. C'est un premier commencement de différenciation. Si chez *Lycaena virgaureae* L. l'apparition d'un nouveau dessin se trouve à ses débuts, chez *Polyommatus daphnis* Den. et Schiff. le phénomène en est à un stade beaucoup plus avancé. Chez cette espèce et surtout chez la femelle, l'habitus est très différent de celui des autres espèces de *Polyommatus*. Cette profonde modification de l'habitus a été à la base de la conception de Sagarra qui a érigé le genre *Meleageria* pour l'espèce *daphnis* Den. et Schiff. Ce genre a été accepté par Forster [1], Verity [11], Beuret [1953] et beaucoup d'autres, mais rejeté par Chapman, Malicky [2] et Stempffer (in litteris). Nous sommes de l'avis des trois derniers auteurs pour la raison que l'armure génitale est de type *Polyommatus*. L'aspect extérieur doit, bien entendu, ne pas être négligé, mais c'est l'armure génitale qui doit avoir prééminence et être considérée

en premier lieu. Pour les espèces, et à plus forte raison pour les genres, le dessin peut être valablement apprécié seulement après l'examen des caractères structuraux et notamment de ceux de l'armure génitale. L'espèce *daphnis* Den. et Schiff. est entrée dans une nouvelle voie, celle d'un nouveau genre, mais pas encore arrivée au point terminus de cette voie. A ces deux exemples on pourrait en ajouter beaucoup d'autres. Ils aboutissent à la même conclusion, à savoir que ces espèces se trouvent, sous nos yeux, en pleine évolution vers de nouveaux genres. Non seulement l'aspect extérieur de l'imago peut nous offrir une indication de ce phénomène, mais aussi les premiers états. Très intéressantes pour le problème qui nous préoccupe sont les modifications qui ont affecté, chez *Lycaena dispar* Haw., tous les premiers états. Ceux-ci diffèrent sensiblement des types de structure des stades respectifs du genre *Lycaena*. Chez toutes les sept espèces de *Lycaena*, mentionnées ci-dessus, le chorion de l'œuf est recouvert d'un réseau polygonal, ces réticulations étant plus ou moins profondes et variables, en ce qui concerne leur forme, suivant les espèces. Le chorion de l'œuf de *Lycaena dispar* Haw. a une toute autre structure. Il est sillonné de six côtes très larges ; dans les espaces intercostaux il y a six rangées de cellules (alvéoles) et dans chaque rangée trois cellules. La chrysalide s'écarte, elle aussi, du type *Lycaena*, étant beaucoup plus grande que chez les autres espèces, et possédant un dessin tout à fait particulier.

Parfois la modification initiale est de nature écologique. Souvent « chez une population qui s'isole d'une autre c'est son genre de vie qui est affecté dès le commencement, soit que la larve s'adapte à une autre plante nourricière, soit que son cycle biologique soit complètement séparé de celui d'une autre population, soit qu'elle s'isole dans un biotope tout à fait différent » [4]. Mais l'adaptation de la chenille à une autre plante-hôte provoque des modifications morphologiques tant chez la chenille que chez l'imago. Ainsi dans le cas de *Lycaena dispar* Haw. on constate un certain particularisme relativement à son écologie, car si les autres espèces de *Lycaena* se nourrissent, d'habitude, avec des feuilles de *Rumex acetosa*, la chenille de *Lycaena dispar* Haw. consomme des feuilles de *Rumex hydrolapathum*. Les particularités morphologiques de la chenille de *Lycaena dispar* Haw. sont, certainement, en rapport avec cette particularité écologique.

Malgré ces différences, *Lycaena dispar* Haw. n'est pas tellement éloignée des autres espèces congénériques pour que nous puissions la considérer comme appartenant à un genre distinct de *Lycaena*. Les modifications ne sont pas encore de nature générique puisqu'elles n'ont pas affecté la sphère génitale — celle qui imprime de la manière la plus prégnante le caractère de genre. L'armure génitale est encore de type *Lycaena*, genre où l'espèce *dispar* Haw. va se maintenir tant que les genitalia ne se modifieront pas. De même que dans le processus de spéciation, ici aussi la modification de la sphère génitale ne se fait pas lentement, mais brusquement, par une mutation. Cette modification sera si puissante que l'armure génitale se réorganisera totalement, faisant apparaître un nouveau type de structure. C'est alors que sera né un nouveau genre, séparé de l'ancien par une discontinuité prégnante — sans formes intermédiaires. C'est seulement alors qu'il sera l'équivalent morphologique et taxonomique de l'ancien genre, mais il aura un autre plan de structure. Le phénomène biologique dont il s'agit peut donc être observé dans la nature dans ses différents stades de développement, les uns à leur début, les autres plus avancés — le tout comme dans le processus de la spéciation [4].

Nous attirons l'attention sur le fait que la réorganisation de l'armure génitale présente, dans la nature, deux aspects : soit au niveau spécifique, soit au niveau générique. Si une race de *Lycaena dispar* Haw. réorganise son armure génitale de telle manière que la nouvelle structure apparue continue d'avoir le même plan, c'est qu'il en est résulté une nouvelle espèce de *Lycaena*. Mais si la réorganisation est plus profonde et la nouvelle structure « sort » du cadre de l'ancien plan, réalisant un plan de structure nouveau, c'est qu'alors il s'agit d'un genre nouveau. Tout dépend par conséquent de l'intensité du phénomène mutationniste qui peut affecter plus ou moins profondément un certain nombre de sclérites. L'intensité du phénomène a une certaine « mesure » pour l'espèce et une autre « mesure » pour le genre.

Imaginons l'apparition d'un nouveau genre auquel donnons le nom de *Lycaenis*. Au début ce genre n'a qu'une seule espèce qui sera nommée, par exemple, *Lycaenis album*. Cette espèce, monotypique, n'a qu'une seule race, à savoir la race nominale : *Lycaenis album album*. Mais, elle devient, au fur et à mesure, polytypique car sur son aire de distribution, qui est de plus en plus vaste, paraissent d'autres races : *Lycaenis album aurata*, *Lycaenis album splendens*, *Lycaenis album purpurascens*, etc. Quand l'une de ces races, par exemple *Lycaenis album purpurascens*, devient par spéciation une nouvelle espèce (*Lycaenis purpurascens*) celle-ci peut être, à son tour, le point de départ d'autres espèces de *Lycaenis*, mais elle peut s'engager aussi sur la voie d'un nouveau genre, par une réorganisation plus profonde de son armure génitale, en réalisant un autre plan de structure de rang générique.

A cette occasion nous soulignons de nouveau l'invalidité des genres *Heodes* Dalman, *Thersamonia* Verity et *Paleochrysophanus* Verity. Le premier a été érigé par Dalman (1816) qui ne connaissait pas l'armure génitale, les deux autres ont été érigés par R. Verity précisément en se basant sur les genitalia. Mais Verity, esprit extrême diviseur, a mal interprété les caractères fournis par l'armure génitale en transférant les caractères spécifiques au niveau générique. Ce point de vue, erroné, a été malheureusement adopté par la plupart des lépidoptéristes, mais à présent il y a une tendance de plus en plus manifeste à abandonner le point de vue extrême diviseur. Pour le cas présent, la conception opposée à celle de Verity a été exprimée par Malicky [2] ; Stempffer, le meilleur connaisseur des *Lycaenidae* du monde entier, a exprimé (in litteris) la même opinion que la nôtre.

Même chose pour *Meleageria* Sagarra dont l'armure génitale est de type *Agrodiaetus* (et *Polyommatus*). Pour justifier le « genre » *Meleageria*, R. Verity [11] le réunit avec le « genre » *Agrodiaetus* Hübner en formant une paire de « genres jumeaux » ! Nous avous montré ailleurs [5] que la notion d'espèces jumelles est un non-sens et la réunion d'espèces proches en « paires » est une opération gratuite. Nous pouvons dire la même chose des genres jumeaux. On pourrait dresser une liste contenant des milliers de « paires » de genres voisins. Que pouvons-nous dire de la paire *Melea-*

geria-Agrodiaetus où aucun des deux « genres » n'a de rang générique? Le fait ne demande pas d'autres commentaires.

De l'exposé ci-dessus il résulte que les diverses particularités morphologiques, qui sembleraient des déviations aberrantes et que nous constatons souvent chez les diverses espèces, sont en réalité des phénomènes normaux qui nous signalent des étapes, soit vers la formation de nouvelles espèces, soit vers la formation de nouveaux genres. Si le spécialiste connaît très bien son groupe et s'il a une vision claire du plan de structure des genres respectifs du groupe, il est capable de discerner ces particularités et de les apprécier judicieusement au niveau spécifique ou générique — suivant le cas.

Ainsi le lépidoptériste, doué d'une conception saine des notions d'espèce et de genre, explique correctement ces faits et les intègre dans le grand phénomène de l'évolution.

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Reçu le 3 avril 1971

NEW AND RARE SPECIES OF FISHES FROM THE PERU-CHILE TRENCH, COLLECTED DURING THE 11TH CRUISE OF R/V "ANTON BRUUN" (1965)

BY

TEODOR T. NALBANT and RUDOLF F. MAYER

Six species of fishes from the Peru-Chile Trench are described, two of which are new: *Bathypterois quadrifilis peruanus* new subspecies and *Menziesichthys bacescui* new genus and new species.

The present contribution is the first of a series of studies of an extensive collection of fishes, obtained and preserved by Dr. Mihai C. Băcescu, during his trip aboard of R/V "Anton Bruun" in the South-East Pacific (Peru-Chile Trench) in the fall of 1965.

More than 600 fish specimens were obtained from the northern to central part of the Peru-Chile Trench (between 3°40′ lat. South and 12°40′ lat. South). The specimens were collected by means of the beam trawl, Menzies trawl and plankton nets (in some plankton stations).

We are particularly indebted to Dr. Mihai C. Băcescu for his kindness in giving us this collection for study. Special thanks go to Dr. Marie Louise Bauchot, Muséum National d'Histoire Naturelle, Paris, Dr. Yvonne Herman, Department of Geology, Washington State University, Pullman, Dr. Jorgen G. Nielsen, Universitetets Zoologiske Museum, Copenhague, Dr. Daniel M. Cohen, U. S. National Museum, Washington, D. C., and to Dr. W. I. Follett, California Academy of Sciences, San Francisco, who provided us with useful informations and reprints.

All specimens, including the types, are deposited in the Natural History Museum fish collection, Bucharest.

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Fam. **BATHYPTEROIDAE**

Bathypterois quadrifilis peruanus subsp. nov.

Fig. 1 a–g

Holotype: Type Fish Collection Cat. Nr. 168, one adult female 115.2 mm in standard length, *Anton Bruun* Expedition, Peru-Chile Trench, October 1965, collected by Dr. Mihai C. Băcescu. No other data. The single specimen known.

Diagnosis. A Bathypterois with characteristic deeply pectinated scales under the base of pectorals, with a very long bifid superior pectoral ray. Preadipose space smaller than in *Bathypterois quadrifilis* Günther.

Description. Body long somewhat compressed, covered by moderate scales. Head depressed, with a large interorbitary space. Eyes small. Mouth cleft large.

The arrangement of genipores: 9 on each side of the mandibula, 7 on each preoperculum, 5 supramaxillars on each side and 6 on each superior part of the head. Two tiny nostrils in front of each eye. Tiny teeth, present on the maxillaries, on both sides of the vomer, palatines, mandibula and on the tip of tongue. Branchial arches reach near the tip of tongue.

The following morphometric features are expressed in percentages of the standard length (in brackets are given the values for the type specimen of *B. quadrifilis* Günther, from Bauchot, 1): head 22.30 (21.40) eye 2.61 (3.33), interorbitary space 7.56 (no data), preorbitary space 6.54 (no data), postorbitary space 13.30 (no data), length of maxillary 13.32 (13.90), greatest height of body 12.20 (12.60), depth of caudal peduncle 7.40 (no data), predorsal space 42.30 (43.80), preventral space 37.00 (38.20), preanal space 57.00 (59.40), preadipose space 72.60 (78.30), longest pectoral ray 97.00 (no data), longest ventral ray 24.75 (no data).

Scales moderate cycloid. Under the base of pectorals 5—6 pectinate scales are visible. The scales in the anterior part of lateral line are slightly pectinated. Under a highly magnifying microscope, (more than 700×) a characteristic structure appears in the subdorsal scales of the lateral line. Some of the circular striae are denticulated, having on the opposite part numerous long setae. The length of denticles varies from 0.2 μ to 0.7 μ while the length of setae varies from 1.2 μ to 1.5 μ. However, this interesting structure does not appear in all circular striae. Scales in lateral line 63 and 62.

The fin rays formula: D 14 +1 (the last ray of the dorsal fin is very close to the 14th ray) A 9; V 9 and 9, the anterior two rays being longer than the others; P 2 +1 +10 in the left fin and 2 +1 +11 in the right fin; C 8 +7 branched rays. The inferior part of insertion of the caudal fin has a peculiar structure as given in Fig. 1 g.

The longest (superior) pectoral rays are fused only in their anterior fourth. Unfortunately, all inferior pectoral rays, in both sides are broken and therefore it is impossible to define their length.

Inner organisation. The stomach is swollen and finishes in a narrow pyloric portion. Pyloric caeca absent. The intestine runs straight up to the anal orifice. The liver is relatively small with a short lobe on the left side. The gall-bladder is developed and detached from the liver. No gas-bladder. The ovaries are long, both having 24.3 mm in length including the ducts. Ovulae relatively large, from 500 to 700 μ in diameter.

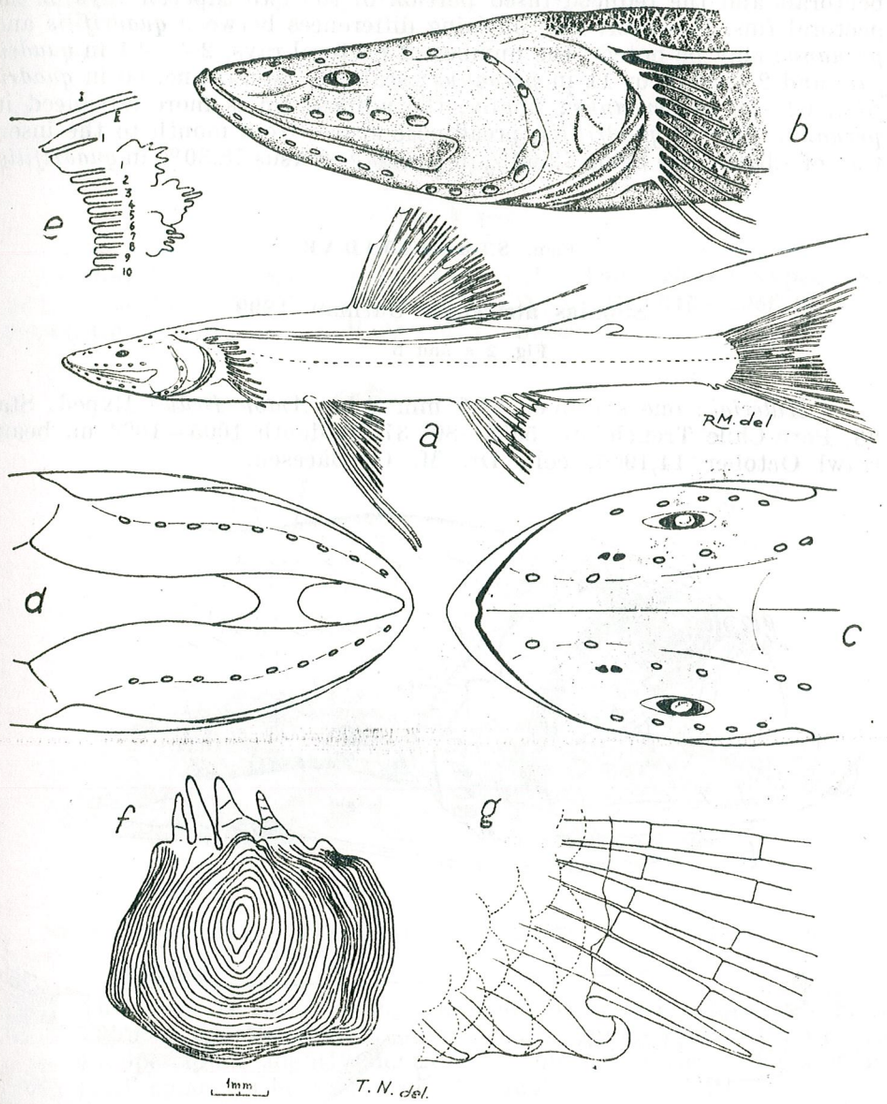

Fig. 1. — *Bathypterois longifilis peruanus* ssp. nov.; a. Outline of the holotype; b. Head lateral; c. Head dorsal; d. Head ventral; e. Details of the pectoral fin with pectinated scales; f. Details of a pectinated scale; g. Inferior part of the caudal insertion. Details.

Colour. Generally dusky brown. Each scale with a white margin. Lateral line marked as a straight white stripe. Maxillaries and the anterior part of the head translucent. Peritoneum deep brown. All fins including filaments, whitish.

Remarks. This new form is similar in some aspects with *B. quadrifilis* Günther, especially as regards the pectinate scales under the base of pectorals and the reduced fused portion of the two superior rays of the pectoral fins. However, the following differences between *quadrifilis* and *peruanus* may be noted : the number of pectoral rays, 2 +8 +1 in *quadrifilis* and 2 +1 +10 or 11 in *peruanus* ; scales in lateral line, 60 in *quadrifilis*, 62—63 in *peruanus* ; finally, the adipose fin is more advanced in *peruanus* than in *quadrifilis*, preadipose space (from mouth to the insertion of adipose fin) being 72.60% in *peruanus* versus 78.30% in *quadrifilis*.

Fam. **STOMIATIDAE**

Stomias atriventer Garman, 1899

Fig. 2 a and b

Material : one specimen 69.9 mm S.L., *Anton Bruun* Exped. Sta. 88, Peru-Chile Trench, 07° 58'S—80° 37'W, depth 1005—1024 m, beam trawl October 14,1965, coll. Dr. M. C. Băcescu.

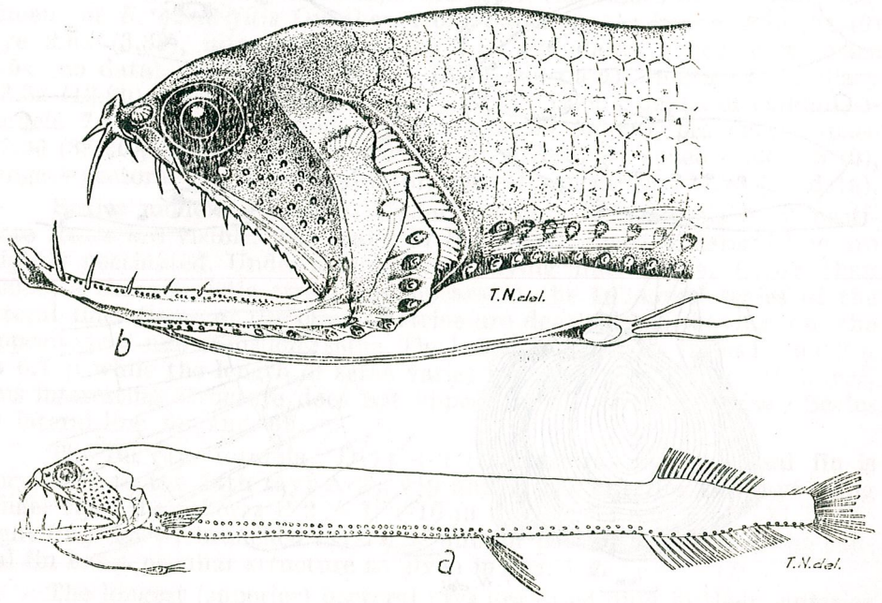

Fig. 2. — *Stomias atriventer* Garman ; a. Outline of the specimen ; b. Lateral view of the head, for the arrangement of photophores and dentition.

The total number of photophores in the ventral row are 81, disposed as follows : 9 on the istmus, 45 from the root of pectorals to the root of ventrals, 12 from the ventrals to the anal origin and 15 from the anal origin to the base of caudal.

Fin rays formula : D 16, A 17, V 5, P 7, C 18.

Remarks. Possibly the 11 specimens of *Stomias boa boa* Risso mentioned by Bussing [3], may represent this species.

Fam. **BROTULIDAE**

Bassogigas sp.

Fig. 3 a—c

Material : one specimen 110.0 mm S.L., *Anton Bruun* Exped., Sta. 161, Peru-Chile Trench, 08°23'S—80°25'W, depth 2945—2966 m, beam trawl, October 31, 1965, Coll. Dr. M. C. Băcescu.

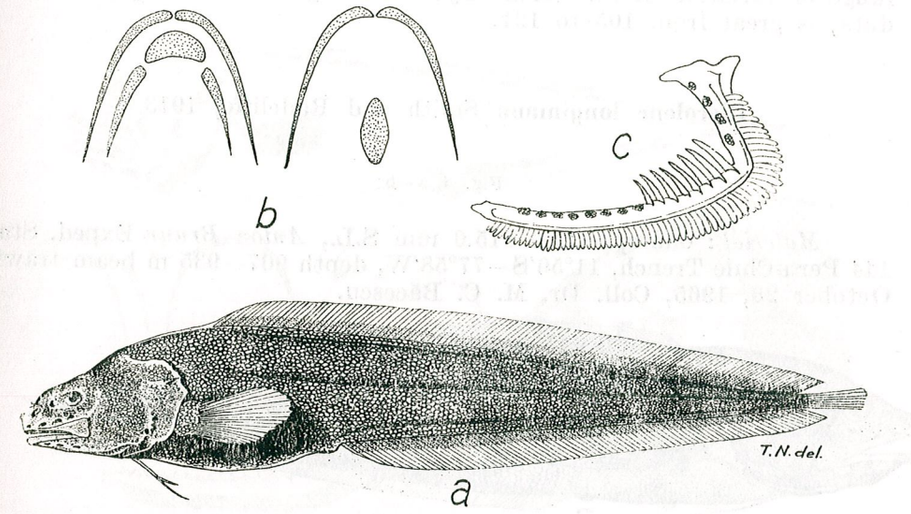

Fig. 3. — *Bassogigas* sp. ; a. Lateral view of the specimen ; b. The arrangement of dentition ; c. The first left branchial arch.

The body is rather elongated and compressed, tappering gently to the caudal fin. Head robust. Eyes relatively small, covered by skin.

Preoperculum slightly denticulated on its vertical edge but not with small spines as in *Neobythites*. Opercular spine robust.

A strong carina between interorbital space and first ray of the dorsal fin.

Gill rakers on the first left branchial arch : 4 small tubercles +8 spines +8 small tubercles (total 20).

Tiny teeth on premaxillaries and palatines. Vomerine teeth arranged on a half-moon shaped plate. An elliptical patch of basibranchial teeth is present at the base of the tongue. Small papillae cover the entire mouth cavity and the tongue. Snout rounded, mouth terminal. Posterior margin of maxillary ends slightly beyond the vertical of the posterior rim of the orbit.

Fin rays formula : D 129, A 99, V 2, P 27, C 4+5 (total 9).

Caudal fin free, not included by dorsal and anal.

Colour : generally gray-violet. Sides of head graysh-brown. A narrow postopercular blackish area. The abdomen is bluish-gray. The mouth cavity, the gill chamber and the peritoneum are deep brown pigmented. All fins pale.

Remarks. The present specimen appears to be distinct from all known species of *Bassogigas* by its greater number of dorsal and pectoral rays, serrated preoperculars and especially by the presence of a strong predorsal keel. However, among the other species of this genus, *B. digitatus* Garman may be considered as the closest relative to our specimen due to its general appearance, by its great number of dorsal rays. The range of variation of the dorsal rays according to Garman [5] meristic data, is great from 105 to 121.

Dicrolene longimana Smith and Radcliffe, 1913

Fig. 4 a—b

Material : one specimen 315.0 mm S.L., *Anton Bruun* Exped. Sta 144 Peru-Chile Trench, 11°50′S—77°58′W, depth 907—935 m beam trawl October 26, 1965, Coll. Dr. M. C. Băcescu.

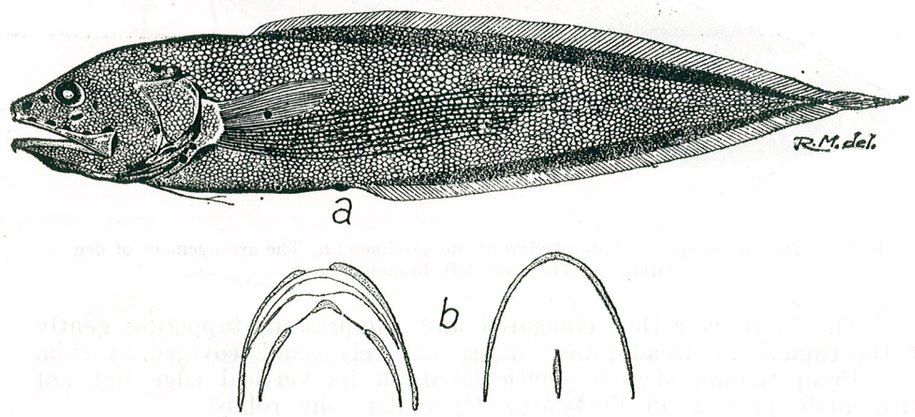

Fig. 4. — *Dicrolene longimana* Smith and Radcliffe; a. Lateral view of the specimen b. The arrangement of dentition.

Fin rays formula : D 111, A 90, V 2, P 13+9, C 4+5. Gill rakers 23, long. Tiny teeth present on maxillaries, vomer and palatines on the upper jaw. In the lower jaw the teeth are present on dentaries and in a narrow patch, on basibranchial.

Remarks. This present species seems to be conspecific with *D. filamentosa* Garman (5). However, our specimen differs from both *longimana* and *filamentosa*, especially by its fin rays formula.

For comparison, see also the original descriptions of these two species with Radcliffe [7] and Garman [5].

Hephthocara sp.

Fig. 5 a—e

Material : one adult specimen 324.0 mm in standard length, *Anton Bruun* Exped., Peru-Chile Trench, October 1965, Coll. Dr. M. C. Băcescu. No other data. One juvenile specimen 64.8 mm in standard length, *Anton Bruun* Exped., Sta 95, Peru-Chile Trench, 08°31′S—81°40′W Menzies trawl, October 15, 1965, Coll. Dr. M. C. Băcescu.

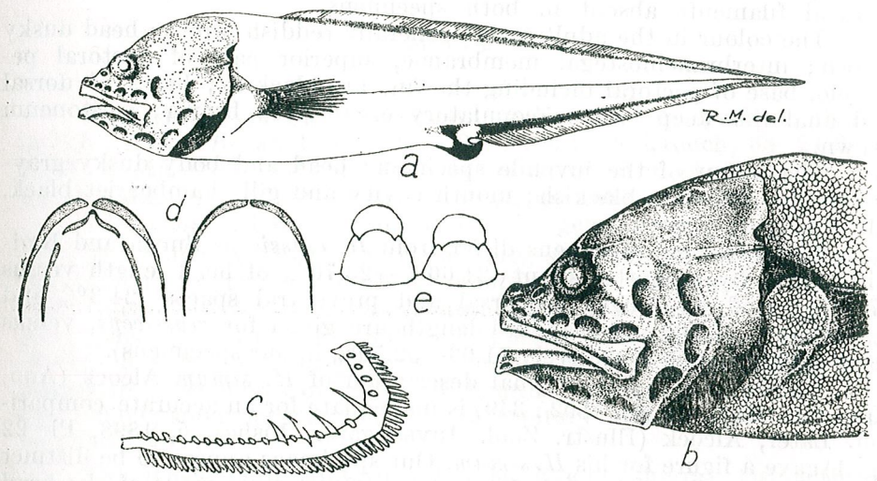

Fig. 5. — *Hephthocara* sp.; a. Outline of the adult male; b. Details of the head; c. The first left branchial arch in the same specimen; d. The arrangement of dentition, same specimen; e. Two chisel-like teeth from the mandible.

The body is long, very much attenuated posteriorly. Head robust and scaleless. The head bones are thin and smooth, being translucent in the juvenile specimen. Gill rakers on the left first arch in the adult male, 22 and 19 in the juvenile specimen. In the longest specimen there are 4 small tubercles (knots), 4 spines (between them 3 small tubercles) and

11 tubercles or knots. In the juvenile specimen, the arrangement of spines is as follow : 4 conspicuous obtuse and spiny knots, 5 denticulated long spines and 11 knots like the first four.

Pseudobranchiae present on the inner face of the operculum, as two minute diverticles. Tiny teeth on premaxillaries, vomer, palatines and dentaries in the juvenile specimen.

The teeth in the adult male are robust at the base, with a chisel like tip ; on vomer and palatines the teeth are arranged in narrow bands. No teeth on basibranchial. Snout blunt, mouth terminal, lower jaw not included. The posterior margin of maxillaries extended beyond the posterior rim of the orbit.

Two nostrils, the posterior one being larger and situated in front of the eye.

In the adult specimen, a series of large pores are distributed as follow (on each side of the head) : supranasal 2, supramaxillary 6, postorbitar 1, preopercular 3, mandibular 4.

In the juvenile specimen only the 4 mandibular pores are discernible.

Preoperculum without spines. Opercular spine weak in both specimens. Body covered by tiny cycloid scales. Whole head naked.

Dorsal and anal continuous with the caudal.

Fin rays formula (in brackets for the juvenile specimen) : D 135 cca (140 cca), A 100 cca (114 cca), P 18 (19), C 4+4 (broken in juvenile). Ventral filaments absent in both specimens.

The colour in the adult male : generally reddish-brown ; head dusky brown ; interbranchiostegal membranae, superior part of pectoral peduncle, base of pectoral including the fin, are blackish ; bases of dorsal and anal fins deep brown ; copulatory organ deep brown ; peritoneum brown.

The colour of the juvenile specimen : head and body dusky gray-brown the abdomen blackish ; mouth cavity and gill chamber jet black. All fins pale brown.

Remarks. Our specimens differ from *H. crassiceps* Smith and Radcliffe, 1913 by a shorter snout (21.60%—23.70% of head length versus 27.70%), and by longer predorsal and preventral spaces (24.3% and respectively 37.30% of standard length are given for *crassiceps*, versus 29.40—30.06% and rspectively 51.03—52.50% in our specimens).

Unfortunately, the original description of *H. simum* Alcock (Ann. Mag. Nat. Hist., *10* (6), 1892 : 349) is inadequate for an accurate comparison. Later, Alcock (Illustr. Zool. Investigator, Fishes, *5*, 1898, Pl. 22 fig. 1) gave a figure for his *H. simum*. Our specimens appear to be distinct from Alcock's *simum* in their head shape, the dorsal profile of the head being in *simum* more abrupt.

Fam. **CYCLOPTERIDAE**

Menziesichthys nov. gen.

Type species : *Menziesichthys bacescui* sp. nov.

Diagnosis. Disk absent. Head large, with a large terminal mouth. Acicular teeth present on dentaries, premaxillaries and vomer.

Eyes hidden under the skin. Nostril single. Two pairs of barbels on the lower jaw, the anterior being the longest. A small protuberance with two strong spines behind the upper part of the operculum.

Description. Body moderately heavy anteriorly, attenuated posteriorly. Head large, compressed, the cheeks are vertical. Mouth cleft large, with both jaws equal. The posterior margin of the mouth under the posterior rim of the orbit. Teeth in narrow bands, acicular, present on the dentaries, maxillaries and vomer. A single nostril on each side of the head in front of the eyes. The first pair of barbels inserted in the middle part of the lower jaw and the second pair inserted at the mouth corner. Eyes large, covered by skin, their contour being hardly discernible. Gill opening generally large, up to the pectoral insertion. Two postopercular spines on each side of the body, projected obliquely upward and downward. Disk absent. The pectoral fin deeply notched. Last vertebra much compressed as a hypural plate. Two pterygophore bones below the hypural. Caudal rays apparently lacking. No prickles on the body.

This genus is named *Menziesichthys*, in honour of Dr. Robert H. Menzies, the head scientist of the 11th cruise of the R/V "Anton Bruun".

Menziesichthys bacescui sp. nova.

Fig. 6 a—f

Holotype : Type Fish Collection Cat. Nr. 169, one specimen 43.2 mm in S.L., *Anton Bruun* Exped. Sta. 94, Peru-Chile Trench, 08° 21′S—81° 25′W, depth 1296—1317 m, Menzies trawl, October 14, 1965, preserved by Dr. Mihai C. Băcescu. Only the type specimen is known.

Diagnosis and description as for the genus.

The following morphometric features are expressed in percentages of the standard length : greatest height (depth) of the body (and of the head) : 19.47 ; head length : 23.20 ; horizontal diameter of the eye : 4.62 ; preorbital space (length of the snout) ; 4.62 ; postorbitary space : 10.40 ; length of the maxillary 11.10 ; predorsal space : 24.30 ; preanal space 30.00. Values in percentages of the head length : horizontal diameter of the eye : 20.00 ; snout length : 20.00 ; postorbitary space : 45.00 ; length of maxillary 48.00.

Colour : generally uniform yellowish. Eye bluish-gray, visible through the transparency of the skin. Abdomen deep gray. Peritoneum blackish. Mouth cavity unpigmented. All fins pale.

Remarks. The new genus and species, appear closely related to *Rhinoliparis*, but differ from it by the presence of two strong spines behind the upper part of the head and by the presence of two pairs of barbels on the lower jaw. For further details in comparison with *Rhinoliparis*, see Burke [2].

This species is named in honour of Dr. Mihai C. Băcescu who collected and preserved this very interesting Liparid fish.

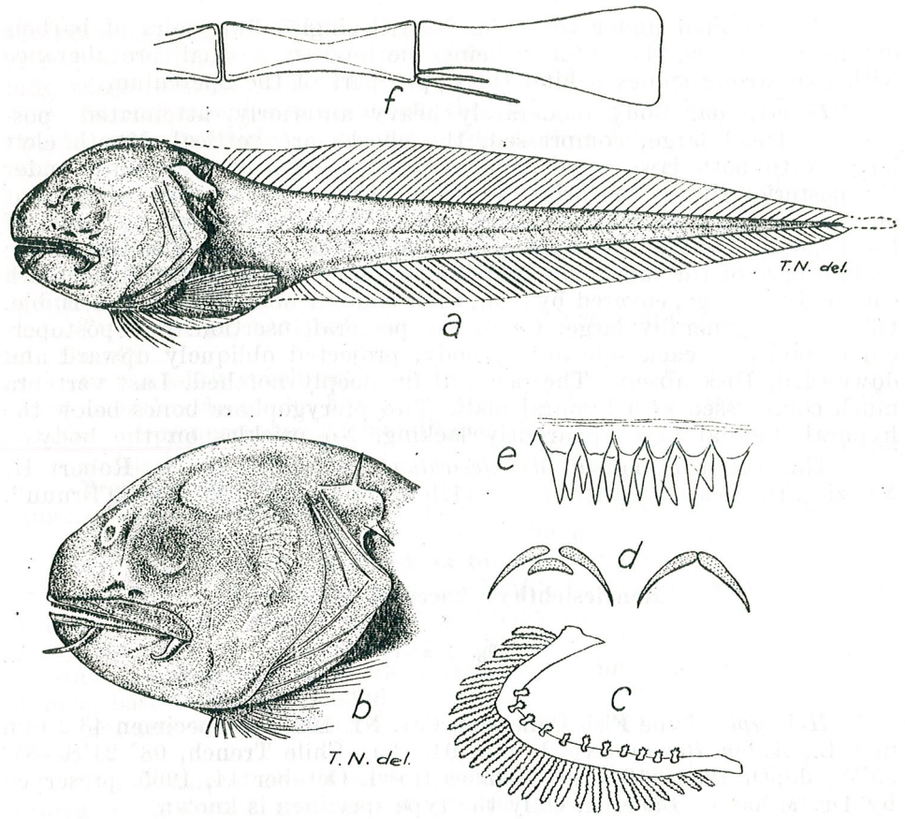

Fig. 6. — *Menziesichthys bacescui* gen. nov., sp. nov; a. Lateral view of the holotype; b. Head, details; c. First right branchial arch; d. The arrangement of dentition; e. Maxillary teeth, detail; f. The last vertebral region.

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The Natural History Museum "Grigore Antipa"
Department of Inferior Vertebrates.

THE REFLECTION OF MEDULLARY HEMATOPOIESIS MODIFICATIONS, SECONDARY TO THE INTERACTION OF THE BIOFIELD WITH THE ARTIFICIAL ELECTROMAGNETIC FIELD ON PERIPHERAL LEUKOGRAM

BY

P. JITARIU, C. SCHNELLER-PAVELESCU and ELENA CHERA

The changes occurring in the medullar hematopoetic activity are evident in the peripheral leukogram in the following way:

— *For leucocytes.* During the first stage of the experiments they generally decrease in the myelogram and increase in the leucogram, while during the second stage they go up compensatorily in the myelogram and go down in the peripheral leukogram.

— *For lymphocytes.* During the first stage of the experiments they decrease sharply in the myelogram and increase in the peripheral leukogram, while during the second stage they increase slightly in the myelogram — but somehow more rapidly than during the first stage in the peripheral leukogram.

— *For monocytes.* During the first stage of the experiments they decrease in the marrow and increase sharply in the peripheral leukogram, while during the second stage a slight increase occurs in the marrow and a very rapid percentage increase in the peripheral leukogram.

All myelogram changes presented in previous works as secondary to the interaction of the biofield with the artificial electromagnetic field are reflected in the peripheral leukogram, whose changes have already been presented in a previous communication.

The evaluation of those modifications has met with difficulties, as the number of cytological species represented in percentage in the leukogram is much lower than that in the myelogram. For this reason, a calculation system appropriate to this end has to be adopted. Myelogram percentages have therefore, been recalculated by us, creating the so-

called "myelogram leukogram" which includes only elements from the leukogram. To this purpose, we have selected elements near or in the last maturation stage. These calculations constituted a whole considered to be 100% and then percentages were recalculated for each cellular species, thus forming the percentage picture of the "myelogram leukogram" or the medullary leukogram.

We present below two tables, i.e. :

Table 1, including numerical values and their variations for each experimental group (control, after 5 experiments, after 10 experiments).

Table 2, including percentage and numerical comparative data of leukocytic cellular elements from the myelogram and leukogram. Calculations were made by the criterion of "myelogram leukogram" as may be easily seen when examining the table.

The analysis of these two tables, especially of Table 1, might help in reaching some conclusions. Yet, the graphic representation of the results helps the reader in drawing some conclusions on the reflection dynamics and in finding an interpretation comparable to that suggested by us.

In the 7 figures presented below, the abscissa records the number of experiments [10]. The whole series of experiments was divided into two stages, i.e. : stage I, ranging between origin and experiment 5, and stage II, ranging between experiment 6 and 10. To better reveal the evolution of the data obtained in the two stages, we transposed the values obtained in stage II on points 5 and 10 of the abscissa. We aimed at graphically illustrating the behaviour of the hematopoietic organ and at reflecting this behaviour at the periphery, in both phases. The 7 figures record numerical data of Table 1.

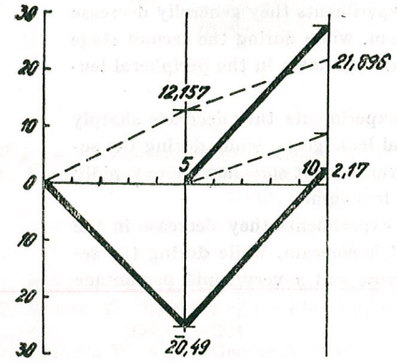

Fig. 1. — ▬ myelogram ; – – – leukogram ; 1 % = 1 mm

Figure 1, reproduced below, represents the way in which peripheral leukograms reflect the variation of adult and almost adult leukocytic elements from the myelogram (Graph 1).

This figure shows that in stage I of our experiments, the cells of the marrow leukocytic series, found in the last maturation stage decrease by 20.49% while they increase by 12.157% in the peripheral leukogram. In stage II, both leukocytic cellular series increase, but while medullary leukocytic cells have a more rapid growth, those of the peripheral leukogram have a milder variation slope. If the starting point of stage II is transposed on point 5 of the abscissa, as may be seen from the graph, the comparative increase rate is pointed out.

This fact as recorded on the graph may be interpreted as follows : in stage I of the experiments, the decrease of medullary elements may result from their increased cytodiabesis, towards the circulating flow, in stage II, both leukocytic series increase, since there occurs a recovery

of medullary cytological activity, attributed by us to the compensatory stimulation, secondary to the interaction of the biofield interaction with the artificial electromagnetic field. The same interaction could occur in the permeability modifications of the membranes, through which these cytological elements pass into the blood flow.

The phenomena have a different aspect when examining graph 2 given below, which presents the way in which the peripheral leukogram reflects the variations of total granulocytes from the medullary hematopoietic tissue (Graph 2).

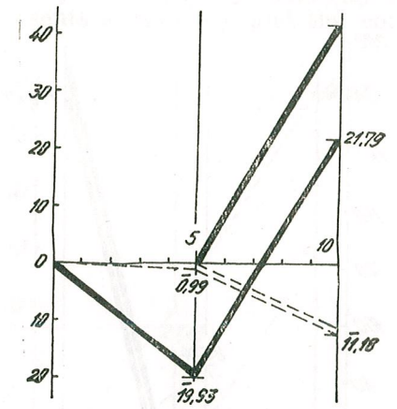

Fig. 2. — ▬ myelogram – – – leukogram ; 1 % = 1 mm

In stage I of the experiments, it is noticed that while medullary cellular elements present a pronounced decrease (—19.93), the leukogram elements vary so little that the state may be considered as stationary.

Stage II presents an obvious recovery of the maturation activity in the hematogenous marrow. In exchange, at the periphery, a marked decrease of total granulocytic leukocytes is observed. In our opinion, in this stage total granulocytes pass into the tissues, which results in their decrease. We adopt this opinion as the hypothesis of granulocyte disappearance cannot be put forward. They will occur later in circulation, according to blood homeostasis.

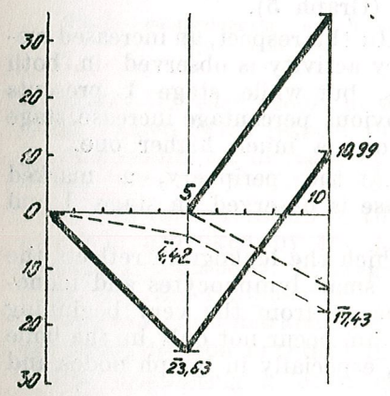

Fig. 3. — ▬ myelogram – – – leukogram ; 1 % = 1 mm

Figure 3 presents the way peripheral leukogram reflects the variation of medullary neutrophil granulocytes (Graph 3).

This graph resembles the previous one, but the decrease of neutrophil granulocytes is more pronounced both in stage I (— 4.42) and in stage II (—17.43).

The discordance between the reaction of hematogenic marrow to the interaction of the e.m. fields studied by us, as well as its reflection at the periphery, can only strenghten our opinion that neutrophil granulocytes pass from the blood into the tissues in a first stage, and then turn back into the general circulation.

We shall now present the peripheral reflection of eosinophil and basophil granulocyte variation. Since these cytological elements are much fewer than the other cytological elements, percentage figures representing them have high deviations and irregular graphs (Graph 4).

Figure 4 presents the peripheral reflection of medularry eosinophil granulocyte variation.

It is noticed that in the first stage of the experiments (1—5) there occurs a decrease, followed by an important increase in stage II. At the same time, however, in the peripheral leukograms there occurs a reverse phenomenon: an important increase in stage I (85.24%), followed by a decrease in stage II.

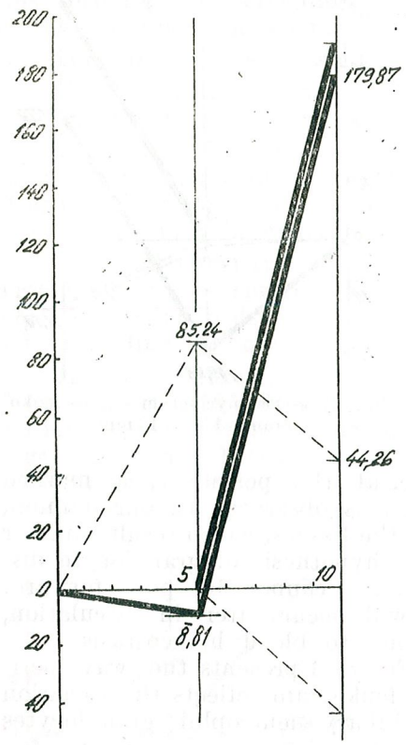

Fig. 4. — ▬ myelogram — — — leukogram; 1% = 0.5 mm

The interpretation is the following: in stage I, the decrease of eosinophil granulocytes from the hematogenic marrow is simultaneous with the return of tissue eosinophil granulocytes into the circulatory flow. Though unequal, this reversion might have a compensatory role. In the second stage there occurs a very pronounced increase of eosinophil granulocytes from the hematogenic marrow, while in the periphery there appears a very marked decrease, probably induced by their passage into the tissues.

Figure 5 presents the peripheral reflection of the medullary activity, as concerns basophil granulocytes (Graph 5).

In this respect, an increased medullary activity is observed in both stages, but while stage I presents an obvious percentage increase, stage II shows a much higher one.

At the periphery, a marked increase is observed in stage I and a slower one in stage II.

We shall now present the way in which the leukogram reflects the cytological variations of the medium and small lymphocytes and monocytes of the myelogram. We must emphasize from the very beginning the fact that these elements of the leukogram occur not only in the bone marrow but also in other multiple tissues, especially in lymph nodes and in the reticulohistiocytic tissue.

In this case we might, therefore, speak of the results of the interaction of these e.m. fields on the whole organism. We have previously shown that in the course of our experiments, the animal is wholly introduced into the artificial e.m. field. As a consequence the results obtained can be interpreted only in the light of the studied interaction and of its effects on marrow and all the other tissues with blood cytological activity, or, to a lesser extent, which have ontogenetic hematological potentialities.

Figure 6, further presented, shows the way in which medullary hematopoiesis modification referring to average of medium and small lymphocytes are reflected at the periphery (Graph 6).

This figure points out that medullary activity very markedly decreases in stage I (—20.73). Stage II presents a recovery and the compensatory increase occurs at a slower rate, so that after 10 experiments only —14.08 (with respect to the control) is reached again. The leukogram presents an increase up to 28.68 in the first stage; in the second stage, however, the increase is even more pronounced (68.08).

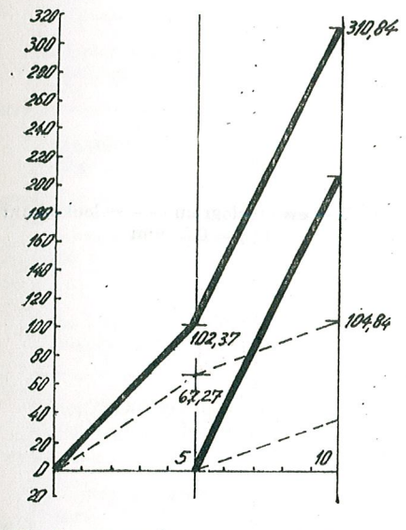

Fig. 5. — ▬ myelogram — — — leukogram; 1% = 0.25 mm

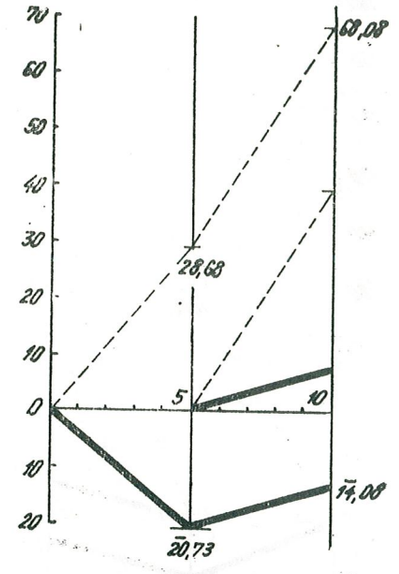

Fig. 6. — ▬ myelogram — — — leukogram 1% = 1 mm

Our interpretation is the following: in stage I, the medullary decrease is provoked by the peripheral increase, due in our opinion, to marked cytodiabesis. In stage II, there occurs a slight medullary recovery, but the lymphocyte increase in the peripheral leukogram occurs at an even more marked rate than in stage I. In our opinion this is also provoked by the entrance into circulation of numerous lymphatic elements, originating in other centres which generate these elements.

Figure 7 shows that the evolution of cytological monocytic medullary and peripheral elements largely occurs after a scheme similar to that of figure 6 (Graph 7).

In this case, since monocytes have a multiple cytological origin, their pronounced increase at the periphery, in comparison with the medullary decrease, can be caused — in our opinion — only by the penetration of monocytic and reticulohistiocytic elements into the circulation.

In general, in our opinion, while medullary cytological activity can be attributed to a complex neuroendocrine apparatus, the reflection of this activity at the periphery depends on membrane permeability,

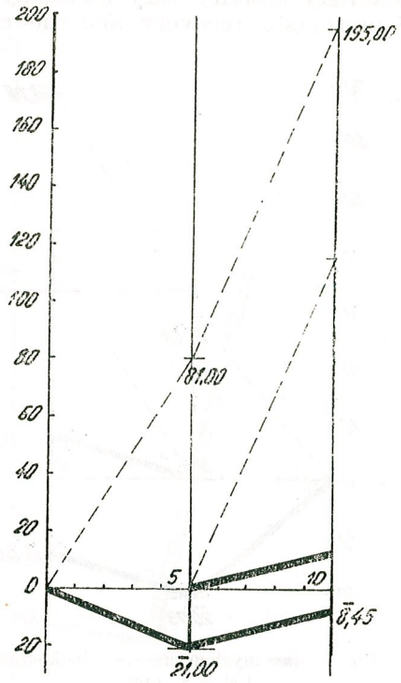

Fig. 7.— ▬ myelogram — — — leukogram
1 % = 0.5 mm

related to the intensity of the cytodiabesis process or to its reverse, the penetration of blood cytological elements into circulation.

DJSCUSSIONS AND FINAL CONCLUSIONS

As already seen, hematopoiesis is a complex and continuous maturation process of some cells with potentialities of their own, ending in the appearance of the adult cell. This occurs in hematopoietic organs, especially in the marrow, lymph nodes, reticulo-histiocytic tissue, etc. In some conditions, especially pathological ones, maturation may occur in organs and tissues which had played a hematopoietic role in the first stages of embryonic life and then had another physiological role. Yet they keep hematopoietic potentialities and may recover them in physiopathological conditions.

Under these conditions, the biofield may play a part in two very important processes, as follows:

1) The biofield of each protein molecule, as well as of the respective cell, has the role of performing the necessary energy transfer from one

Table 1

Numerical values and their variations

No	Category of cytological elements		C	5	10
1	Elements of the myelogram leukocytic series in the last maturation stage	No/cmm	26526	21091	27100
		variation	0	$\overline{20}$.49	2.17
	Elements of peripheral leukogram	No/cmm	7024	7878	8562
		variation	0	12.157	21.896
2	Granulocytic elements of the myelogram	No/cmm	10671	8545	10671
		variation	0	$\overline{19}$.93	21.79
	Granulocytic elements of the leukogram	No/cmm	4186	4174	3696
		variation	0	$\overline{0}$.99	$\overline{11}$,18
3	Neutrophil granulocytic elements of the myelogram	No/cmm	10217	7803	11340
		variation	0	$\overline{23}$.63	10.99
	Neutrophil granulocytic elements of the leukogram	No/cmm	3960	3785	3270
		variation	0	$\overline{4}$.42	$\overline{17}$.43
4	Eosinophilic granulocytic elements of the myelogram	No/cmm	159	145	445
		variation	0	$\overline{8}$.81	179.87
	Eosinophilic granulocytic elements of the leukogram	No/cmm	61	113	88
		variation	0	85.24	44.26
5	Basophil granulocytic elements of the myelogram	No/cmm	295	597	1212
		variation	0	102.37	310.84
	Basophil granulocytic elements of the leukogram	No/cmm	165	276	338
		variation	0	67.27	104.84
6	Average and small lymphocytes of the myelogram	No/cmm	7334	5814	6302
		variation	0	$\overline{20}$.73	$\overline{14}$.08
	Average and small lymphocytes of the leukogram	No/cmm	2703	3477	4542
		variation	0	28.68	68.08
7	Monocytic cells of the myelogram	No/cmm	8521	6732	7801
		variation	0	$\overline{21}$.00	$\overline{8}$.45
	Monocytic cells of the leukogram	No/cmm	100	181	295
		variation	0	81	195

Table 2

Table of comparative percentage and numerical data of leucocytic cytological elements in the myelogram and leukogram

No		C		5		10	
1	Total number of nucleated cellular elements/cmm in the myelopoietic tissue	79633		76500		87166	
2	Total number of segmented granulocytic elements in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		33.31	26526	27.57	21 091	31.09	27100
3	Total number of segmented neutrophil granulocytes in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		38.51	10217	36.99	7803	41.84	11340
4	Total number of segmented eosinophil granulocytes in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		0.59	159	0.68	145	1.64	445
5	Total number of segmented basophil granulocytes in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		1.11	295	2.83	597	4.47	1212
6	Total number of average and small lymphocytes in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		27.64	7334	27.56	5814	23.21	6302
7	Total number of monocytic cells in the myelogram	%	No/cmm	%	No/cmm	%	No/cmm
		32.12	8521	39.91	6732	28.78	7801
	Σ	≈100	26526	≈100	≈21091	≈100	≈27100
8	Total number of peripheral leukocytes	7024	var. %	7878	var. % 12.158	8562	Var. % 21.896
9	Total number of neutrophil peripheral leukocytes	%	No/cmm	%	No/cmm	%	No/cmm
		56.375	3960	48.039	3785	38.118	3270
10	Total number of eosinophil peripheral leukocytes	%	No/cmm	%	No/cmm	%	No/cmm
		0.875	61	1.434	113	1.026	88
11	Total number of basophil peripheral leukocytes	%	No/cmm	%	No/cmm	%	No/cmm
		2.350	165	3.500	276	3.947	338
12	Total number of leukogram lymphocytes	%	No/cmm	%	No/cmm	%	No/cmm
		38.475	2702	44.131	3477	53.052	4542
13	Leukogram monocytes	%	No/cmm	%	No/cm	%	No/cmm
		1.425	100	2.302	181	3.447	295
	Σ	≈100	≈ 7024	≈ 100	≈ 7878	≈100	≈ 8562

element to the other, to ensure the course of the chain enzymatic reaction, according to a well established programme.

2) This energy transmission which plays a part in starting every process from the long reaction series should be strictly codified, since otherwise maturation does not result in a normal adult cell.

The interaction of the biofield with the artificial electromagnetic field may influence the maturation process under discussion by an energetic exchange. The modality of interaction occurrence is not yet known. Data obtained by us seem to point to a maturation acceleration, but we consider that in other experimental conditions, an opposite result may be obtained. This opinion is based on the fact that the parameters of the artificial electromagnetic field should correspond as much as possible to the biofield parameters and we first refer to frequency; in this case, we think that a resonance-analogous phenomenon could be obtained, hence, results might be much more significant than those obtained so far. For the time being, biofield parameters are infinitely few and experiments liable to capture and amplify it are not yet perfected.

We consider that the biofield is locally generated by the protein molecule on the glucolipoproteic complex, according to the physiological necessity of the moment. These complex structures, where a ceaseless movement of charged particles occurs, produce the biofield which continuously vary. This variation may occur in the following conditions:

— first spontaneously, better said from unknown causes, resulting from the physiological requirements of the moment;

— second, due to the biofield of neighbouring structural elements;

— third, because of biocurrents produced by the nervous influx of small fibres in the immediate neighbourhood or in direct relationship with cytological elements of the respective tissue. In this case, one may pose the problem of energy contribution or of energy transfer.

The artificial electromagnetic field is interacting with the whole organism, but this interaction varies from organ to organ, from tissue to tissue. In this case, interaction is directly proportional — in our opinion — to the resonance degree obtained. In our case, the hematopoietic apparatus which has one of the most intense cytological activity and is considered as an "always young tissue", has a wider biofield than other organs, proportionately speaking. Besides, this high variability enables the occurrence of a resonance approach from time to time. This accounts for the higher sensitivity of the hematopoietic tissue to the interaction with the artificial electromagnetic field.

Hematopoiesis is, however, a process which occurs in many organs and tissues and, hence, largely possesses its own homeostasis. Reference is first made to the balance between the production of specific blood cells in the respective organs and the removal from circulation of elements rendered useless by "wearing". We refer particularly to the normal physiological conditions and from this standpoint we observe that the myelogram includes only young elements, since adult or almost adult elements pass through cytodiabesis into the general circulation. We then notice that between the cytological structure of the myelogram and of the leukogram, a certain ratio is maintained which varies only within certain

limits, which are exceeded only in pathological conditions. The above facts may lead to the conclusion that hematopoietic activity, no matter whether it occurs in organs or tissues, possesses a complex control mechanism [2] [3]. This problem was posed so far by relatively few authors, but in the case of our experiments we need a scheme of this mechanism to work with. Subsequently, we or others might change it in relation with the conclusions drawn from the respective experiments. In the scheme proposed below we must say from the very beginning that the activity of marrow and of the other hematopoietic organs is not directly linked with the peripheral tissues or the leukogram.

We consider from the leukogram structure that the presence of blood elements in the tissues is decisive for the activity of hematopoietic organs though not directly but by the intermediary of the diencephalon centres, which receive information under the form of impulses directly from the periphery, on pathways and relays incompletely elucidated from an anatomical standpoint. In this way, an integration of information occurs in these centres and a conclusion is drawn on the blood cells requirements of the organism which are permanently changing. When these facts are known by the cortex, the activity tonus of subjacent centres is modified by the cortex. The centres of the diencephalon are also involved, wherefrom necessary impulses are starting, either for the activation or for the reduction of the activity of hematopoietic organs and tissues. As a result, the latter should yield blood cell elements meeting the present needs of the organism. It is necessary to add that the peripheral leukogram does not reflect as a whole the organism requirement of blood cells but, in our opinion, gives only some indications, as a large part of cells are passing from the circulation into the tissues.

Within the framework of our experiments, we think that the biofield of living structures is involved. Yet, one must first pose the problem of the interaction of microfields, since two neighbouring microfields may have a very slight interaction, because of the high difference between the respective frequencies or they may have a higher interaction, as the frequency difference decreases.

As for the interaction of the biofield with the artificial electromagnetic field, the problem might be posed in two ways :

— the artificial electromagnetic field might interact with each biological microfield, and the interaction could vary according to the degree of the obtained resonance from case to case ;

— the artificial electromagnetic field might interact with the microfield resultant, in other words with the tissue or organ biofield. Experiments were not yet performed, as no appropriate techniques are available. Data representing the biofield parameters are, therefore, not available either.

The importance of knowing biofield parameters is very great for our experiments, as this knowledge could lead us to the utilisation of an artificial electromagnetic field, liable to come into resonance with the biofield. For this reason, the parameters of the artificial field used by us were randomly determined.

The way our experiments were carried out makes us consider them as a simple, initial scheme, necessary only at this stage of our studies. Yet, if we analyse more carefully this question, we must notice that there are other factors still, which not only have not been taken into account, but have not even been mentioned. In further experiments these factors should be given due consideration and they may even enter calculations, which are now possible thanks to modern techniques. In our studies on biofield interaction with the artificial electromagnetic field we have considered only the electromagnetic field fully described by Maxwell's equations.

Of the factors not mentioned by us, we shall recall only atomic nuclei, with nuclear magnetic moments. These nuclei may emit quantas of varied frequencies. We took this fact into account as it is easy to understand, but the existence of an almost infinite number of nucle iin living structures makes it difficult to admit the hypothesis that they do not interfere with life processes, sometimes even resulting in pathological phenomena.

Hematopoiesis was studied by us in the rabbit, a higher mammal with a complex biological activity. The study of the biofield interaction with the artificial electromagnetic field by following up medullary hematopoiesis changes led us to notice the following effects :

— changes of maturation dynamics, generally higher in the myelogram cellular series ;

— changes of the maturation rate, increased in the leukocytic series and decrease in the erythrocytic one ;

— decrease of the cytological interdependence of the medullary series proving that the myelogram, in normal physiological conditions, is forming a unitary whole, almost as an organ with its own role. These experiments approaching a very complex biological phenomenon — hematopoiesis — had not the advantage of appropriate techniques. However, this question, though simply treated, poses several fundamental problems for present-day physiology which shall have very important consequences in human physiology and physiopathology.

We shall mention below only three problems which seem more important :

1) The problem of structure and of the phenomenon. These two entities form a whole, since a certain structure is connected only with the occurrence of certain phenomena. Therefore, the elucidation of biological structures, which is being carried on, will result in the explanation of certain phenomena and, hence, will be really useful for physiology — — the more so since, in our opinion, physiology could also be called "structure biophenomenology".

2) The problem of bioenergy and of its distribution. This involves the biofield, closely related both to structures and to the modality of its distribution or, briefly, to its quantification.

3) The problem of radiation codifying within complex phenomena of the living organism, with a very important role in biological automatism.

A more careful examination of the above problems makes us find out that they are but different facets of one and the same problem, which we might designate by a single word: *life*. And all the other questions which have not been mentioned derive from these three problems.

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The Centre of Biological Researches
Yassy

TETRAXIN ACTION ON RAT ADENOHYPOPHYSIS

BY

ELENA HEFCO, CSÖNGE BRANDSCH and P. JITARIU

Anterior pituitary weight changes and the histological picture of the gland after tetraxin administration were followed.

No modification in pituitary weight was observed, but a very significant increase of eosinophil percentage and a decrease of both basophils and chromophobes percentage were registered.

In a previous paper [3] we reported that after the administration of 200 parts per million (ppm) tetraxin to rats weighig 35—50 g., for four weeks, we obtained a significant gain in weight in the treated animals. We tried to see if the drug used did not exert its action through the anterior pituitary, stimulating the growth hormone elaboration.

For this purpose, we determined the anterior pituitary weight and followed the histological changes in the gland, ascertaining the percentage of acidophil, basophil and chromophobe cells.

MATERIALS AND METHODS

Male Wistar rats, weighing 35—50 g., fed on a complete diet according to McCollum [10] and water ad libium, were used. The animals were divided into two groups: one of controls fed on a standard diet and the second one, treated, to whose diet 200 ppm tetraxin (calculated to tetracycline content) were added. After administering, for four weeks the biostimulator, the animals were sacrificed and the pituitaries were removed. For histological study, the glands were fixed in Bouin-Hollande-sublimate and stained by the M. H.-II teracrom met thod [5].

The percentage of adenohypophyseal cells was determined by counting a thousand cells per gland.

The significance of the differences between the groups was determined by Student's metest.

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RESULTS AND DISCUSSION

Anterior pituitary weight (Table 1) expressed either on an absolute weight basis, or relative to body weight (mg./100 g.b.w.) was unaffected after four weeks of tetraxin administration. The fact is not surprising because anterior pituitary is a gland with weight changes which poorly reflect or do not reflect at all the modifications of its activity [4].

Table 1

Anterior pituitary weight after tetraxin administration

Group	Number of rats	Anterior pituitary weight(mg)	Relative anterior pituitary weight (mg./100 g.b.w.)
Control	18	4.2±0.20	3.5±0.15
Tetraxin	24	4.5±0.12	3.5±0.14
		N.S.	N.S.

Values are mean ± S.E
N.S. – non-significant

Following the histological picture, in all cases, we observed that the percentage of acidophil cells in the tetraxin group presented a more significant increase than in the control group. Chromophobe and basophil cells were fewer in the treated animals than in controls (Table 2).

Table 2

Histological picture of adenohypophysis after tetraxin administration

Group	Number of rats	Eosinophils %	Basophils %	Chromophobes %
Control	14	49.9±0.9	17.7±0.7	32.3±0.7
Tetraxin	14	60.9±0.6	15.1±0.4	23.9±0.4
		p<0.001	p<0.001	p<0.001

Since acidophils are known as a source of growth hormone (GH), the very significant increased number of these cells may be considered to determine an increased synthesis and release of GH, fact that explains the gain in body weight observed in animals fed tetraxin, and the other biochemical modifications previously reported [3].

The decreased percentage of chromophobes is explainable on the one hand, because it is known that these cells represent the precursors of active cells [11], in other words, a conversion of chromophobes into eosinophils occurred. On the other hand, some authors conside rthe chromophobes as the site of ACTH formation [7] hormone with catabolic action.

The body weight changes are a result of both anabolic and catabolic hormone actions, and the preponderence of one of these determine

an increase, respectively, a decrease in body weight [1]. A diminished number of basophils, cells responsible for TSH secretion, indicates a decreased activity of other catabolic hormone. In consequence, a preponderence of GH action results.

These histological data, correlated with gain in body weight, slight decrease of plasma amino nitrogen and muscular mass gain-well known as a result of GH action upon body metabolism-obtained after tetraxin administration in rats, are indices which attest an increase of both GH synthesis and release, due to the biostimulator.

Similar histological modifications have been reported by Ozawa [12] in rabbits, after CTC administration (20 mg). The author suggests a hyperfunction of the anterior lobe of the hypophysis due to the antibiotic.

About the question, if this antibiotic action is a direct one, or if it is secondary to the intestinal one, there have been many discussions.

Some data plead for a direct action. Thus Bruggemann [2] has reported that daily parenteral administration of antibiotics to rats promoted growth similar to that produced by oral administration in the species.

A potentiating action on anabolic reaction via GH or a retardation of the function of catabolic regulators such as cortical steroids [13] or thyroxine [2] has also been proposed. This fact is attested by the decreased number of basophils responsible for TSH elaboration.

The circumstance that the antibiotics act as stabilizers in stress conditions [6], that they stimulate the growth of "germfree" chicks and turkeys [8], [9] are proofs which plead for a direct action of antibiotics upon the animals.

The big number of papers reporting on the antibiotic effect upon ntestinal microflora, plead for an indirect effect of antibiotics as growth stimulators; this effect cannot be overlooked.

Therefore, it is more valid to say that the antibiotic growth effect is a very complex one and represents the result of several actions including the prevention and alleviation of "sub-clinic" infections, intestinal synthesis, increased appetite, adaptation response and action through the hormones.

CONCLUSIONS

1. Tetraxin administration to weanling rats does not produce anterior pituitary weight modification.

2. Significant changes in the adenohypophyseal cell percentage (increase of eosinophils and the decrease of basophils and chromophobes) were observed.

3. There is an increased GH secretion responsible for weight gain in animals.

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Center of Biological Research
Department of Physiology,
Yassy

EFFECT OF MONOFLUORACETATE AND MALONATE ON THE CYTOPLASMIC NAD-LINKED METABOLIC SYSTEMS IN RAT LIVER *IN VIVO*

BY

I. V. DEACIUC and GH. FRECUŞ

Blood glucose level and the content of lactate, pyruvate, glycerol 1-phosphate (GIP), dihydroxyacetone phosphate (DAP), malate, oxaloacetate (OXAC) and glycogen in liver were determined in rats after monofluoracetate (MFA) and malonate treatment. Both inhibitors induced a hyperglycemia but did not affect the content of liver glycogen. Lactate to pyruvate ratio increased after both MFA and malonate treatment, while GIP to DAP ratio increased only after malonate injection. In no case were changes in malate to OXAC ratio observed.

Experimental data were reported in the literature showing that the inhibition of the citric acid cycle activity with monofluoracetate (MFA) and malonate leads to metabolic alterations, some of which are common with those observed in diabetic organism. Among them, the most striking ones in respect to their similarity with those in diabetes are hyperglycemia and hyperketonemia, observed in rabbits and rats after MFA ([2], [6], [19]) or malonate ([9],[18], [20]) administration. Another metabolic alteration common to the diabetic organism and to that treated with MFA is the increase in the citrate content of tissues. This has been demonstrated in diabetic animals ([8], [28], [30]) as well as in those treated with MFA ([5], [17], [30]). Although caused by different mechanisms, the increase of the citrate content has common metabolic consequences, among which of a special interest is the inhibition of the phosphofructokinase activity ([15], [28], [29]) and, therefore, the depression of glycolysis.

On the other hand, there are experimental data suggesting that in the diabetic state, the activity of the citric acid cycle is impaired ([24],

[32]). It has been considered that this impairment is one of the key metabolic events responsible for hyperglycemia and, in part, for hyperketonemia. If so, one may expect that the inhibition of the citric acid cycle would lead to other metabolic alterations seen in diabetic organism too.

In the present paper an attempt was made to study the effect of MFA and malonate, administered *in vivo*, on the redox state of three cytoplasmic NAD-linked metabolic systems, by measuring the content of corresponding intermediates and the reducant/oxidant ratio.

MATERIALS AND METHODS

Chemicals. These were from the following sources: NAD, oxidized, from E. Merck AG, Darmstadt, Germany; NADH, from Nutritional Biochemicals,. Ohio. USA; malate dehydrogenase (crystalline, from pig heart, in ammonium sulfate suspension), from C. F. Beohringer und Soehne, Mannheim, Germany; lactate dehydrogenase and glycerol 1-phosphate dehydrogenase (both crystalline, from rabbit skeletal muscle, in ammonium sulfate suspension) were kindly supplied to us by Dr. F. P. Martynenko from the Institute of Endocrinology and Metabolism, Kiev, USSR. All other chemicals were of highest purity and commercially available.

Treatment of animals, preparation of liver extracts and assay of intermediates. Experiments were performed on female white rats, weighing 160—240 g and maintained on a Larsen diet. Six hours prior to experiment the animals were deprived of food but not of water. MFA-treated animals received the poison in a dose of 6 mg per kg b.w. (0.20 ml of 0.3% Na—MFA in 0.9% NaCl/100 g b.w., intraperitoneally) and 100 min. later, they were injected nembutal (50 mg per kg b.w.). Malonate-treated animals received 2.2 g of Na-malonate per kg b.w. (1.0 ml of 22% Na-malonate, adjusted to pH 7.4, per 100 g b.w.) and, 70 min. later, they were injected nembutal as stated above. The control animals received 1.0 ml of 0.9% NaCl/100 g b.w. and, 100 min. later, they were injected nembutal as above. Twenty min. after nembutal injection in all groups, the abdomen of animals was carefully opened and the liver, so exposed, was clamped between two aluminium blocks (1×4×6 cm) precooled in liquid nitrogen. The tissue pressed between the blocks (its thickness was allways below 2 mm) was immediately immersed, together with the blocks, in liquid nitrogen and then transferred into a porcelain mortar, containing liquid nitrogen. The powder obtained by pulverizing the tissue was extracted with cold $HClO_4$ 6% (w/v) (5.25 ml per 1 g of powder) in all-glass Potter-Elvehjem homogenizer at 0°C. After centrifugation at 0°C, the supernatant fluid was quantitatively tranferred into a centrifuge tube and neutralized at 0—4°C to pH 4.5—5.0 with 5M K_2CO_3. The extract was allowed to remain at 0—4°C for 15—20 min. and then centrifuged. On appropriate aliquots of this extract lactate, pyruvate, glycerol 1-phosphate (GlP), dihydroxyacetone phosphate (DAP), malate, and exaloacetate (OXAC) were assayed enzymatically according to the standard published methods [3], by measuring the changes in optical density at 340 mμ in a Zeiss VSU-1 spectrophotometer, at room temperature.

The glycogen content of the liver was determined on a piece (100—150 mg) of clamped liver; after being weighed on a torsion balance, the piece was immediately plunged in 1 ml of warm 30% (w/v) KOH. Glycogen was isolated according to the procedure of Good, Kramer and Somogyi [16], and assayed by the method of Montgomery [25].

Blood glucose was assayed according to Nelson [26]; the blood was collected from the tail at 10—15 min. after nembutal injection.

The statistical treatment of the data was made according to Student's *t* test. P values of 0.05 or less are considered to be significant.

RESULTS

The data in Table 1 show that both poisons induced a significant increase in blood glucose level (by 110% in the case of MFA, and by 82% in the case of malonate), without affecting the content of liver glycogen.

Table 1

The effect of monofluoracetate and malonate on the blood glucose and liver glygocen in rats

Treatment of animals	Blood glucose (mg/100 ml)	Liver glycogen (%)
Control	103±6 (6)	0.99±0.14 (7)
Monofluoracetate	213±9* (12)	0.81±0.05 (12)
Malonate	188±19* (7)	0.72±0.19 (6)

Values in the Table are mean ± standard error of mean. Number of animals is given in brackets.* Significant $p < 0.01$ for the difference between the control and the treated animals.

In Table 2 data are presented showing the *in vivo* effect of both poisons on the content of intermediates of three NAD-linked metabolic systems in rat liver, as well as on the ratio reductant/oxidant of the corresponding pairs. It is pertinent to point out that the values of intermediate contents reported for the control animals in this paper compare well with those reported by others [1], [21].

Table 2

The effect of monofluoracetate and malonate on the content of some reduced or oxidized NAD-linked intermediates in rat liver *in vivo*

Intermediates and the reduced/oxidized ratio	Treatment of animals		
	Control (9)	Monofluoracetate	Malonate (7)
Lactate	1181±84	2 560±137* (12)	2 532±262*
Pyruvate	114±7	61±4* (12)	82±6*
Lactate / Pyruvate	10.4±0.5	55.9±5.1* (12)	30.7±2.1
GlP	240±28	374±52* (9)	191±41*
DAP	27±3	40±5* (9)	10±2*
GlP / DAP	9.1±0.5	8.6±0.9 (9)	25.6±7.1*
Malate	373±27	566±31 (9)	328±39
OXAC	6±0.6	11±1.6* (9)	6±0.7
Malate / OXAC	67±8.6	56±7.7 (9)	50±3.0

The content of intermediates is expressed as mμmoles/g tissue wet weight (mean ± standard error of mean). The number of animals is given in parantheses.* Signifies a $p < 0.05$ for the difference between the control and the treated animals. For abbreviations, see the text.

As seen in Table 2, MFA treatment resulted in a large increase of the lactate content of the liver; this was associated with a decrease of the pyruvate content and with a rise in the lactate to pyruvate ratio.

The content of both GlP and DAP changed in the same direction, i.e. increased, and by the same order of magnitute so that the ratio GIP/ /DAP remained unaffected. Finally, MFA rose the content of both malate and OXAC, but only the content of the latter was significantly increased. The malate to OXAC ratio was not affected, as revealed by the statistical treatment of the data.

Treatment of animals with malonate led to changes in the lactate and pyruvate content and in their ratio similar to those observed in MFA-treated animals. The level of both GlP and DAP was diminished, but their ratio was markedly increased by malonate. No effect of this poison on malate and OXAC content and on their ratio was observed.

DISCUSSION

Hyperglycemia induced by MFA and malonate, observed by others [2], [6], [19], [20], is confirmed by our results. In addition, we observed that it is not associated with a decrease in liver glycogen content, suggesting that it does not originate by split of liver glycogen. Two mechanisms may be considered as being responsible for hyperglycemia in poisoned animals : an impairment of glucose utilization by the tissues poisoned with MFA and malonate, and an increase of gluconeogenesis in liver or kidney cortex.

The first one appears to be most plausible, at least in the case of MFA, since citrate, which accumulates in different tissues after MFA injection [5], [17], [30], is an inhibitor of phosphofructokinase activity [15], [28], [29] which, in turn, is the rate limiting step in glycolysis. The inhibition of this enzyme results in a rise of glucose 6-phosphate content in tissues [4], [27], [33] and, subsequently, in inhibition of hexokinase activity [7], [31]; finally, a diminution of glucose uptake takes place. These sequential modifications, however, cannot be responsible for hyperglycemia observed in malonate-treated animals. In this case an inhibitory effect of malonate, exerted directly on glycolysis as it has been shown for skeletal muscle *in vitro* [12], might be involved, but this point requires further investigation.

The second mechanism here discussed to explain hyperglycemia in MFA and malonate-treated animals, i.e. an increase in gluconeogenesis, was suggested (but not demonstrated) on the basis of some experimental data obtained *in vivo* on rabbits [19]. However, some of our recent results, obtained in experiments *in vitro* on kidney cortex (to be published by the authors) show that malonate and MFA are strong inhibitors of gluconeogenesis. These data suggest that an increase in gluconeogenesis appears not to be a factor responsible for hyperglycemia in poisoned animals. In connection with these it must also be noted that gluconeogenesis is an ATP consumming process and that ATP regeneration is depressed in liver mitochondria on incubation with MFA [10], [11] or in liver *in vivo* after malonate treatment [23].

It is well documented that in the liver of diabetic animals the cytoplasmic NAD-linked metabolic systems is shifted towards a more reduced state [22]. Our results show that this situation may only in part be

reproduced by the inhibition of the citric acid cycle. It is interesting to note that, unlike the diabetic state in which the values of the reductant to oxidant ratio of the three metabolite pairs move in parallel [22], in animals poisoned with MFA and malonate this is not observed. Thus, after MFA injection a change was observed only for the lactate to pyruvate ratio, while after malonate treatment lactate to pyruvate and GlP to DAP ratios increased simultaneously. This state may be regarded as being a result of disturbance of the tight coupling of NAD-linked reactions in the cytoplasmic compartment of the liver by the inhibitors. The mechanism by which this is achieved remains unknown.

The increase in the content of GlP and DAP seen after MFA injection (Table 2) was unexpected, since inhibition of phosphofructokinase activity would be associated with a decrease in the content of all glycolytic intermediates beginning with fructose 1,6-diphosphate. It is possible that GlP would be formed under these circumstances by pathways others than glycolysis, e.g. by lipolysis from glycerides.

The two inhibitors used in our experiments act on the citric acid cycle at different steps and, in addition, at least one of them, i.e. MFA, is metabolized by pathways giving fluorinated compounds which inhibit other reactions beside that catalyzed by aconitase [13], [14]. Hence, their effect on the redox state of liver cytoplasm may be only in part attributed to their inhibitory effect on the citric acid cycle activity. However, at least the increase in the lactate to pyruvate ratio, seen after both MFA and malonate treatment of animals may be considered as a consequence of the citric acid cycle inhibition.

Although we cannot offer at present an explanation for the mechanism of all effects of the two inhibitors on the redox state in liver cytoplasm, it appears that in diabetic animals the disturbance of the citric acid cycle is only in part responsible for displacement of the redox state of NAD-linked cytoplasmic reactions.

CONCLUSIONS

1. Hyperglycemia seen in rats after MFA or malonate injection is not associated with a decrease in liver glycogen content, suggesting that it does not originate in hepatic glycogenolysis.

2. Inhibition of the citric acid cycle with MFA or malonate leads to a displacement of NAD-linked metabolite systems in liver cytoplasm towards a more reduced state. This is manifested especially by the increase of lactate to pyruvate ratio.

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Biological Research Centre, Cluj
Department of Animal Physiology

CHROMOSOME COMPLEMENT AND SYSTEMATIC POSITION OF A ROMANIAN SPECIES OF THE GENUS *PITYMYS*

BY

P. RAICU and DOINA DUMA

Our cytogenetic studies dealing with two Romanian populations of *Pitymys* lead to the following conclusions:

1. Both populations studied show identical chromosome complements, distinct from that of other species of *Pitymys*, except *P. subterraneus*. It is reasonable to conclude that they form a single subspecies, *P. subterraneus ssp. dacius* (*G. S. Miller*, 1908) with 2n=52.

2. A comparison of the chromosome complements in different species of *Pitymys* revealed the occurrence of highly variable NF values, which support the idea of an ancestral speciation event, which did not proceed only through chromosome rearrangements of the Robertsonian type.

Several conflicting points of view occur concerning the systematics of the Pitymys genus, as different authors increase or decrease the number of species belonging to this genus, based mainly upon morphologic and ecologic investigations. Recent cytogenetical studies succeeded in a more precise delimitation of the species from a taxonomic point of view, by investigating the characters of the chromosome set. Thus, *R. Matthey* (1964) and *A. Meylan* (1970) identified within this genus the following species, bearing different chromosome complements: *P. mariae*, *P. duodecimcostatus* and *P. pinetorum* with 2n = 62, *P. savii* with 2n = 54, *P. subterraneus* with 2n = 52, 53, 54, *P. multiplex* and *P. m. fatioi* with 2n = 48, and *P. tatricus* with 2n = 32. The caryotype diversity displayed by the species of this genus leads to the conclusion that they are the result of an ancient speciation event and form valid species.

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Previous investigations revealed the occurrence of several species of this genus in Romania, such as: *P. dacius* and *P. subterraneus* (*G. S. Miller*, 1908, 1912), *P. transsylvanicus*, *P. nyerensis* and *P. klozeli* (*G. Ehik* 1924, 1942), while *M. Hamar* (1960) and *M. Hamar* and *F. Theiss* (1961) claimed the existence of only one species: *P. subterraneus*.

MATERIAL AND MAETHODS

Male and female individuals of *Pitymys* captured in the Carpathians were used in this study. The animals were obtained from Dr. M. Hamar, chief of the Laboratory for Rodents of the Institute foı Plant Protection.

The animals were colchicinized 1 $^1/_2$ hours prior to sacrifice with a 0.05% colchicine solution, amounting to 1 ml. per 100 gms body weight. After killing the animals, thigh bone marrow and testes for the males were removed and hypotonic treatment was carried out in 0.8% sodium citrate solution at 37^{0}C for 40 minutes. The material was fixed in a 3 : 1 mixture of ethanol and glacial acetic acid and centrifuged several times. Smears were made on well defatted slides. Staining was performed in 10% Giemsa solution for 15 minutes.

RESULTS AND DISCUSSIONS

Both *Pitymys* populations so far examined show identical chromosome complements, consisting in 26 pairs of chromosomes (2n = 52), among which the heterosomes are represented by a big metacentric X chromosome and a small acrocentric Y chromosome. The autosomes are represented by 22 pairs of acrocentrics, one pair of big subtelocentrics, one pair of big submetacentrics and one pair of very small metacentrics (Figs. 1 and 2).

Karyotype study in the two populations of *Pitymys* from Romania reveals no difference as against the species *P. subterraneus* which has 2n = 52, 53, 54 chromosomes, and shows a Robertsonian chromosomal polymorphism.

On the other hand, the karyotype in these populations differs from that of other species of the genus *Pitymys*, cytogenetically studied as yet both as to the chromosome number and the fundamental number of arms (NF), except for *P. subterraneus*.

P. mariae	2n = 62 NF = 72
P. duodecimcostatus and P. pinetorum	2n = 62 NF = 64
P. savii	2n = 54 NF = 62
P. subterraneus	2n = 52, 53, 54 NF = 60
P. multiplex and P. m. fatioi	2n = 48 NF = 54
P. tatricus	2n = 32 NF = 46

The material studied from our country has 2n = 52 and NF = 60 (Graph No. 1).

The variation of the fundamental number of arms (NF) shows that the karyotype diversification within the genus *Pitymys* proceeded

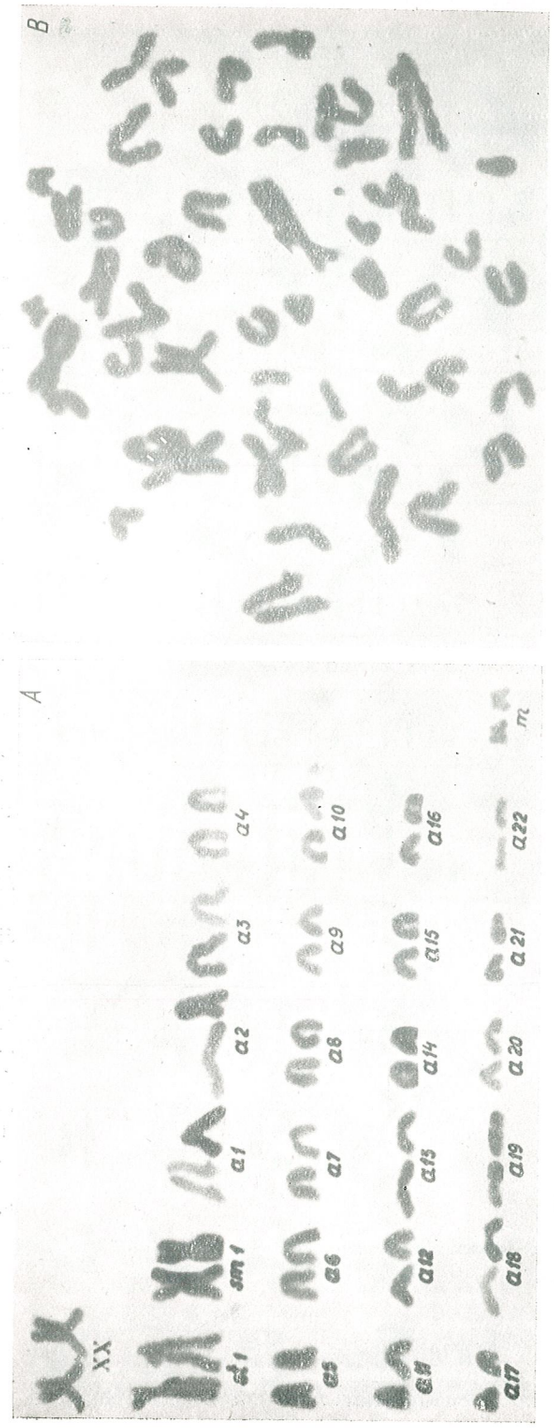


Fig. 1. — Female karyotype in *Pitymys subterraneus ssp. dacius* (A) and their metaphase plate (B).

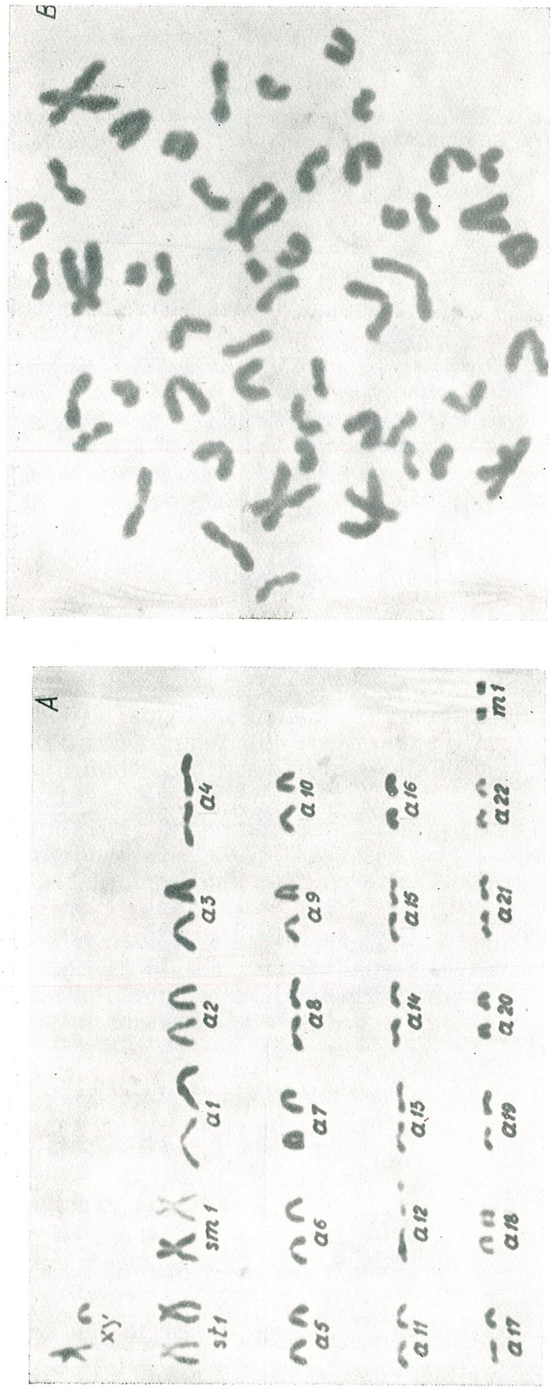
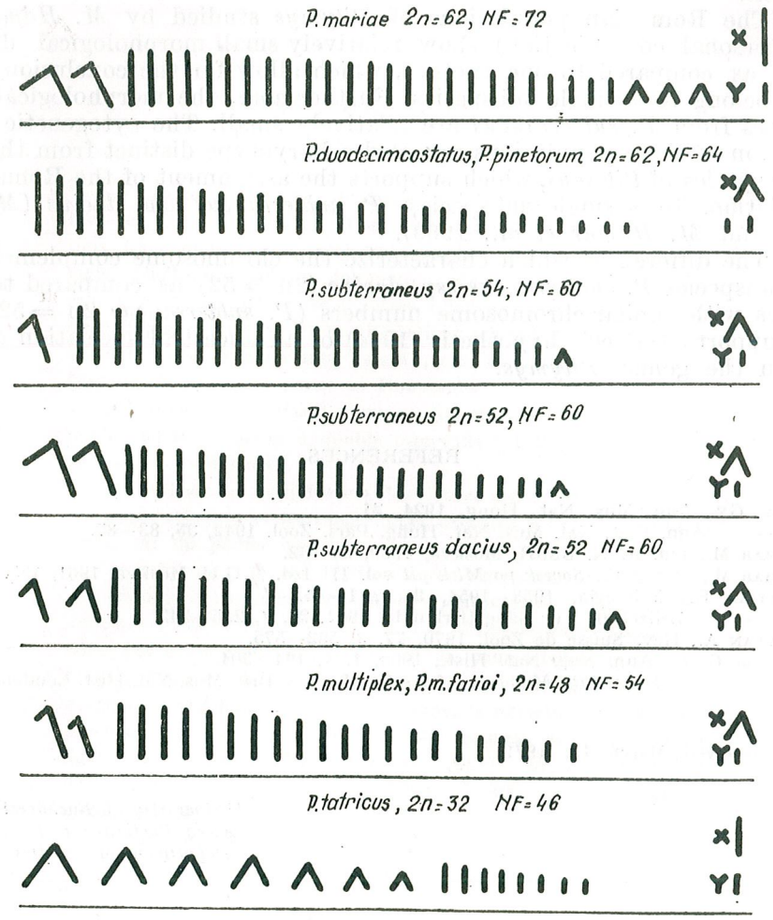


Fig. 2. — Male karyotype in *Pitymys subterraneus ssp. dacius* (A) and their metaphase plate (B).



Graph 1. — Schematical illustration of **chromosomal complement in different** species of *Pitymys*.

not only through Robertsonian mechanisms of the centromere fusion-fission type, but through other types of chromosome rearrangements as well.

The Romanian populations of *Pitymys* studied by *M. Hamar et al.* (personal comunication) show relatively small morphological differences as compared to one another, which allow for the conclusion that they belong to a single subspecies. Furthermore, the morphological differences from *P. subterraneus* are relatively small. The cytogenetic examination of these populations revealed a karyotype distinct from that of other species of *Pitymys*, which supports the assignment of the Romanian populations to a single subspecies, *P. subterraneus ssp. dacius* (*Miller*, 1908 and *M. Hamar et al.*, 1969).

The differences which characterize the chromosome complement of the subspecies *P. subterraneus ssp. dacius* (2n = 52) as compared to the species with similar chromosome numbers (*P. subterraneus* 2n = 52, 53, 54) support *Matthey's* hypothesis (1964) of an ancestral speciation event within the genus *Pitymys*.

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Universitiy of Bucharest
Aleea Portocalilor 1—3
Department of Genetics

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