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SOMMAIRE

DOINA CODREANU-BĂLCESCU, Sur quelques nouvelles espèces du genre *Cephaloidophora,* Grégarines (Protozoa, Apicomplexa) parasites des Amphipodes ponto-caspiens de Roumanie 3

ANDY Z. LEHRER, Distinction taxonomique entre les espèces paléarctiques *Liosarcophaga aegyptica* (Salem) et *Liosarcophaga parkeri* (Rohdendorf) et description de deux espèces nouvelles afrotropicales (Diptera, *Sarcophagidae*) 11

ZOHAIR I. F. RAHEMO and SULAIMAN N. AMI, *Microtetramers* sp. larvae (Nematoda : Tropisuridae) encysted in the stomach wall of *toad, Bufo viridis* in Iraq 19

GR. MIHĂESCU, C. DELCEA, L. GAVRILĂ, Electron microscopical study of methanogene bacteria isolated from Techirghiol lake 25

MARIA NĂSTĂSESCU, VIORICA MANOLACHE, OTILIA ZĂRNESCU, CĂLIN TESIO, The action of mercury on the digestive tract at *Anodonta cygnea piscinalis* (Lamellibranchiata=Bivalvia) 33

ELENA POPOVICI, ANCA-PETRESCU RAIANU and R. POPA, The dynamics of adrenal-medullary catecholamine content following unique aminophylline (theophylline-ethylene-diamine) administration in mice 39

VIORICA VINTILĂ, RODICA GIURGEA, CORINA ROŞIORU and IOANA ROMAN, The action of some resin extracts on the Walker carcinosarcoma in Wistar rats 51

J. MADAR, NINA ŞILDAN, DANIELA BOCHIŞ, G. FRECUŞ, C. PUICĂ and I. ILYÉS, Hepatic glucose overproduction and other endocrine-metabolic disorders in fluocinolone-induced hyperglycemic Wistar rats 55

RODICA GIURGEA and IOANA ROMAN, The manganese effects upon some hepatic biochemical parameters in chickens 61

IOANA ROMAN, RODICA GIURGEA, Z. URAY and MARTA GABOS, Biochemical modifications in the thymus, thyroid and adrenals following a thyroxine and leucotrofina treatment in the female Wistar rats 65
DOINA IONICĂ, Energetical budget of the bacterioplankton in lake Dâmbovița 69
LILIANA GREGORIAN, ATENA SCRIPCARIU, I. R. CIOBANU and P. RAICU, Comparative study for establishing some genetic markers in the autochthonous carp breeds "Frăsinet" and "Ropșa" (*Cyprinus carpio* L.) 73

SUR QUELQUES NOUVELLES ESPÈCES DU GENRE *CEPHALOIDOPHORA*, GRÉGARINES (PROTOZOA, APICOMPLEXA) PARASITES DES AMPHIPODES PONTO-CASPINES DE ROUMANIE

DOINA CODREANU-BĂLCESCU

Six new species of Gregarinida (Protozoa, Apicomplexa) belonging to the genus *Cephaloidophora* Mavrodiadi are described, namely: *C.mucronata* n. sp., *C.similis* n. sp., *C.corophii* n. sp., *C.rotunda* n. sp., *C.elongata* n. sp. and *C.parva* n. sp., all intestinal parasites from Pontocaspian relict Amphipods in the Danube Delta and littoral area of the Black Sea in Romania. The Amphipod genera *Chaetogammarus, Dikerogammarus, Corophium* and the species *Gammarus duebeni* and *G.equicauda* are reported for the first time as hosts for Gregarinida. The affinity of the new-determined species to the *Cephaloidophora* spp. parasitising marine Amphipods proves once more the marine origin of fresh-water Pontocaspian Amphipods.

Quant aux Grégarines parasites des amphipodes relictes ponto-caspiens, outre l'espèce *Uradiophora ramosa* Bălcescu-Codreanu 1974, que nous avons trouvée dans l'intestin moyen de *Pontogammarus robustoides* provenant de «Ghiolul Roșu» dans le Delta du Danube (2), on ne possède plus que de brèves mentions signalant la présence des espèces indéterminées rapportées aux genres *Heliospora* et *Cephaloidophora* chez *Pontogammarus robustoides* et *P.crassus*, provenant du Dniepr et graduellement acclimatés dans les bassins avoisinant la Mer Baltique (6).

Dans le présent travail nous ajutons de nouveaux genres-hôtes, tels *Chaetogammarus, Dikerogammarus, Corophium,* aussi bien que deux espèces du genre *Gammarus,* provenant du benthos de la partie inférieure du Danube (le Delta) et de la zone littorale de la Mer Noire. En examinant leurs intestins nous y avons trouvé six espèces nouvelles de Grégarines inféodées au genre *Cephaloidophora* Mavrodiadi 1908, que nous décrivons ci-dessous.

Les grégarines dégagées de l'intestin des hôtes après dissection en solution de Ringer, ont été examinées sur le vivant, ou fixées aux vapeurs osmiques, ensuite mesurées, dessinées à la chambre claire et photographiées en contraste de phase. Nous avons également utilisé des frottis humides, fixés au liquide Duboscq-Brasil et colorés au hématoxyline-érythrosine.

1. **Cephaloidophora mucronata** n. sp.

Station de récolte: dragages sur le fond sableux-pierreux, près de l'embouchure du bras Sulina (Delta du Danube), mille 0-5.

Hôtes: *Pontogammarus robustoides aestuarius* Derjavin 1924
Chaetogammarus tenellus behningi Martinov 1919
Dikerogammarus haemobaphes fluviatilis Martinov 1919

REV. ROUM. BIOL.–BIOL. ANIM., TOME 40, N° 1, P. 3–10, BUCAREST, 1995

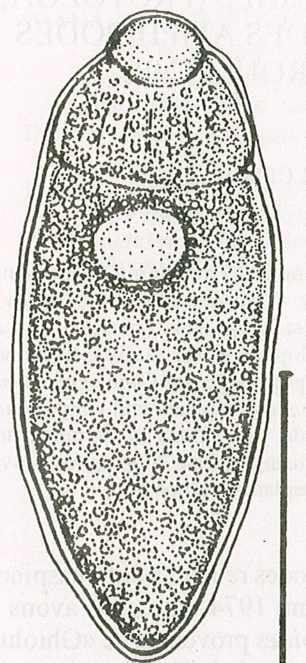

Fig. 1

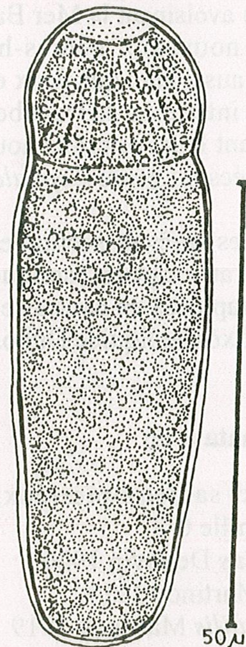

Fig. 2

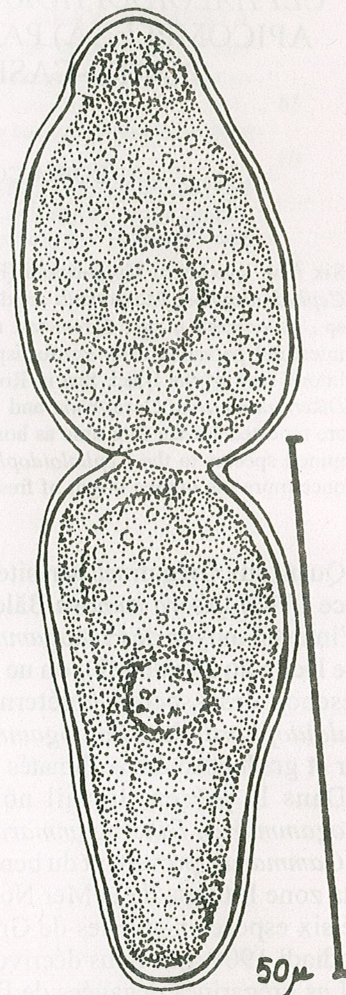

Fig. 3

Fig.1.*Cephaloidophora mucronata* n. sp.-trophozoïte adulte solitaire.
Fig.2.*Cephaloidophora simillis* n. sp.-céphalin solitaire.
Fig.3.*Cephaloidophora corophii* n. sp.-syzygie fronto-caudale.

Localisation: l'intestin antérieur et moyen

Les plus jeunes stades observés sont des céphalins libres dans l'intestin antérieur, ayant une forme allongée et très peu d'inclusions, d'une longueur de 20-30 μ sur 10 μ de largeur maximum. Ils présentent un mouvement oscillatoire et la plupart s'associent de bonne heure en syzygies fronto-caudales, les dimensions des partenaires des plus petits couples correspondant à celles mentionnées ci-dessus. Les gamontes continuent leur croissance conjugués en syzygies, dont la longueur totale varie de 70-130 μ; le primite se montre souvent un peu plus large que le satellite. Les trophozoïtes adultes solitaires (fig.1) ou associés mesurent chacun 55-95 μ et nous donnons ci-après les indices biométriques des gamontes associés en syzygies, caractéristiques pour cette espèce:

TL	LM	LP	LD	LP:TL	WP	WD	WP:WD	N
83μ	7,5 μ	12 μ	64 μ	1:6,8	24 μ	31 μ	1:1,29	12,5μ

Quant à leur conformation (fig.1), ils ont l'aspect général fusiforme, portant à l'extrémité antérieure un mucron globuleux, hyalin, saillissant d'un protomérite bas par rapport au deutomérite; celui-ci, en forme d'ovale-allongée, s'amincit vers l'extrémité caudale. Le noyau arrondi est situé plus ou moins dans la proximité du septum proto-deutoméritique, convexe de son côté.

Dans l'intestin postérieur on peut rencontrer de rares syzygies, mélangées au detritus alimentaire, et qui se préparent à l'enkystement (avec leurs partenaires tassés, 27 μ de diamètre chacun), même que des kystes jeunes, sphériques-ovales, mesurant 29-32 μ × 35-48 μ de diamètre; ils ont encore les deux noyaux distincts (∅ = 8μ).

2. **Cephaloidophora similis** n. sp.

Station de récolte: la même que pour l'espèce précédente
Hôtes: *Chaetogammarus tenellus behningi* Martinov 1919
Dikerogammarus haemobaphes fluviatilis Martinov 1919
Localisation: l'intestin médium

La forme et les dimensions des céphalins solitaires rencontrés (fig.2) sont appropriés aux celles des gamonts associés en syzygies. D'une longueur totale moyenne d'environ 130 μ, ces syzygies peuvent avoir le primite plus long que le satellite. Les moyennes des dimensions des trophozoïtes adultes (sporadins) caractèrisant l'espèce sont:

TL	LM	LP	LD	LP:TL	WP	WD	WP:WD	N
74μ	4,5μ	17μ	53μ	1:4,3	23μ	24μ	1:1,05	12,5μ

Le contour rectangulaire-allongé leur donne une configuration générale plus étroite par rapport à l'espèce précédente (fig.2). Le protomérite plus haut, presque carré, porte un mucron applati, lenticulaire. De la même largeur que le protomérite, le deutomérite, légèrement rétréci dans son quart inférieur, a l'extrémité caudale tronquée, parfois avec une petite excavation médiane.

Bien que cette espèce ait deux hôtes communes avec la précédente, nous n'avons jamais rencontré les deux espèces de grégarines simultanément dans le même individu d'amphipode-hôte.

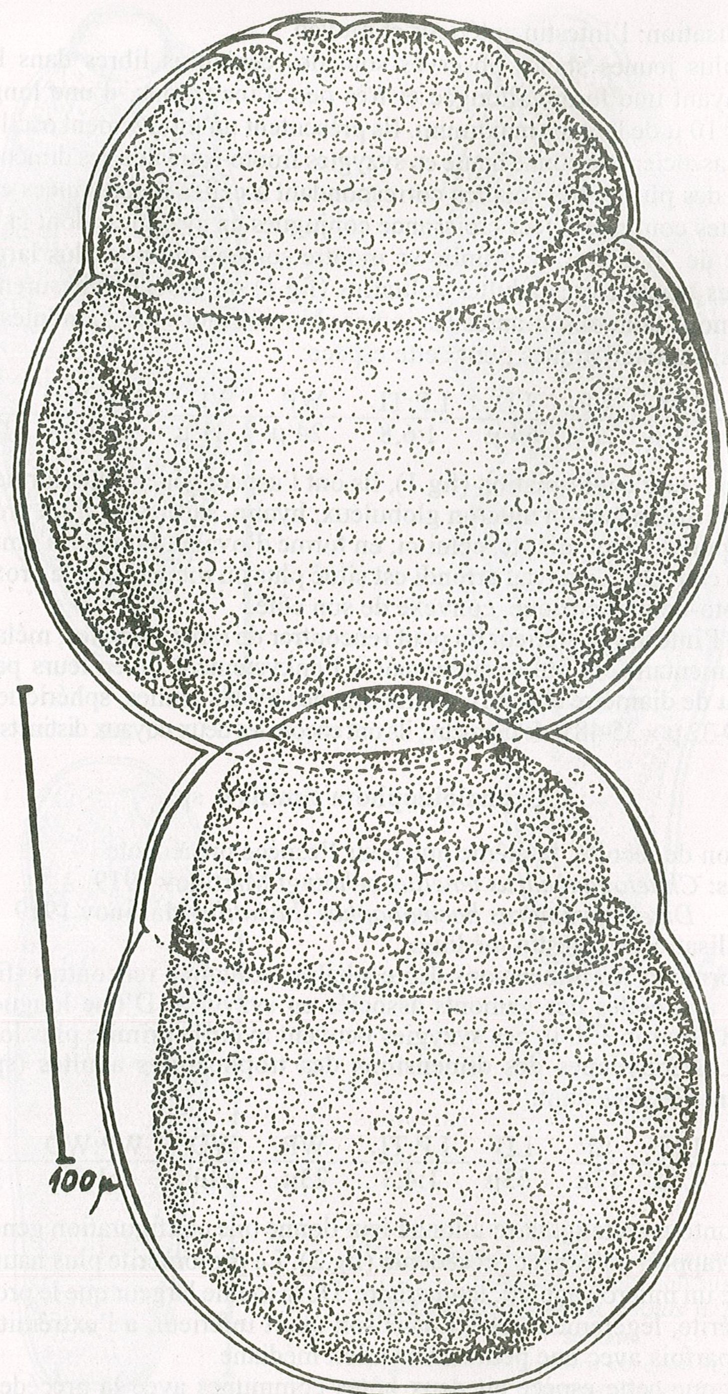

Fig. 4. *Cephaloidophora rotunda* n. sp.-syzygie fronto-caudale.

3. **Cephaloidophora corophii** n. sp.

Station de récolte: ramassé sur les racines de roseau au bord de «Ghiolul Roşu», étang dans le Delta du Danube

Hôte: *Corophium curvispinum* G. O. Sars 1895

Localisation: l'intestin moyen et postérieur

Les syzygies fronto-caudales (fig.3), d'une longueur totale d'environ 90-115 µ, sont constituées des gamonts inégaux comme taille, ayant des aspects dissemblables. Les primites d'une forme générale ovoïde, dilatée postérieurement («en grain de citrouille») diffèrent des satellites plus allongés, avec la largeur maximale dans leur partie antérieure, tandis que celle postérieure s'atténue nettement.

Les gamonts du couple ont le protomérite petit, en voûte, plus étroit que le deutomérite; celui-ci, ovoïdal chez le primite, prend l'aspect général de langue rétrécie vers l'extrémité caudale. Le noyau vésiculeux est placé au centre du deutomérite.

Les indices morphométriques de cette espèce sont:

TL	LM	LD	LP:TL	WP	WD	WP:WD	N
45µ	8µ	37µ	1:5,6	10µ	25µ	1:2,5	7-8µ

4. **Cephaloidophora rotunda** n. sp.

Station de récolte: le facies pierreux au bord du lac littoral Siut-Ghiol, Mamaia, Mer Noire

Hôte: *Corophium maeoticum* Sov. 1898

Localisation: l'intestin moyen

Les syzygies contenant des gamontes globuleux, presqu'égales (fig.4) mesurent en tout 200-300 m. Le protomérite coupuliforme constitue un tiers de la longueur totale du sporadin, étant limité par un septum, dont la convexité se trouve du côté du deutomérite sphérique. Un mucron lenticulaire, applati, s'enfonce dans le deutomérite du primite, assurant la conjugaison des partenaires en syzygies.

Les caractères biométriques des gamontes sont:

TL	LP	LD	LP:TL	WP	WD	WP:WD	N
155µ	60µ	95µ	1:2,7	115µ	150µ	1:1,3	24µ

5. **Cephaloidophora elongata** n. sp.

Station de récolte: les rhisomes de roseau au rivage du lac Belona Eforie-Nord, Mer Noire

Hôte: *Gammarus duebeni* Lilljeborg 1851

Localisation: l'intestin moyen et postérieur

Le taux d'infection assez élevé, 25% des gammares prospectés portant des pelotons des grégarines obstruant la lumière intestinale.

Les plus jeunes stades observés sont des sporadins solitaires et étroits, à contour rectangulaire-allongé (16-27 µ de long sur 7 µ de largeur). Ils s'associent très tôt en syzygies fronto-caudales, en continuant leur croissance jusqu'à une longueur totale de la syzygie d'environ 60-185 µ (fig.5). De temps en temps, le primite peut porter à la fois deux satellites, beaucoup plus petits.

Le protomérite bien représenté a une largeur un peu inférieure à celle du

Fig.5.*Cephaloidophora elongata* n. sp. - syzygie fronto- caudale.

Fig.6.*Cephaloidophora parva* n. sp. -syzygie fronto-caudale.

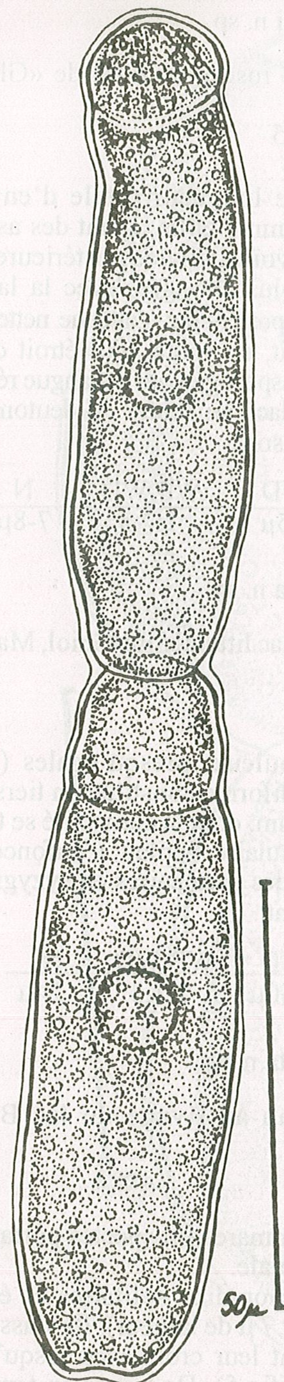

Fig. 5

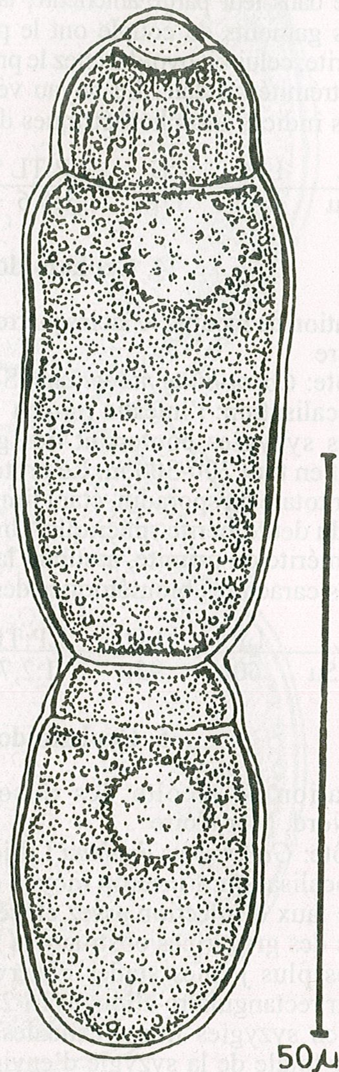

Fig. 6

deutomérite, qui a son tour, présente une forme allongée, uniformément calibrée et tronquée ou légérement escavée à l'extremité postérieure. Le noyau sphérique se trouve vers le centre du deutomérite.

Les indices biométriques pour les gamonts d'une syzygie adulte:

TL	LP	LD	LP:TL	WP	WD	WP:WD	N
85μ	17μ	68μ	1:5	18μ	24μ	1:1,3	10μ

6. Cephaloidophora parva n. sp.

Station de récolte: le cordon formé d'algues littorales dans la zone du bris des vagues contre le rivage de la Mer Noire, plage de Mamaia

Hôte: *Gammarus equicauda* Martinov 1931

Localisation: l'intestin moyen

Le pourcentage général d'infection relativement élevé (30%).

On rencontre de jeunes céphalins isolés, de 30-35 μ longueur sur 13 μ largeur, munis antérieurement d'un mucron saillant, ainsi que des syzygies d'une longueur totale moyenne d'env. 80-100 μ (fig.6). Il y a des cas où le satellite est plus petit que le primite. Les gamonts ont l'aspect général rectangulaire avec les coins arrondis; le deutomérite ovalaire-allongé se sépare par un septum rectiligne du protomérite bas porteur d'un mucron convexe, bien représenté. Les noyaux globuleux excentriques, placés juste au dessous du septum proto-deutoméritique.

Les indices biométriques pour les gamontes adultes sont:

TL	LP	LD	LP:TL	WP	WD	WP:WD	N
50μ	12μ	38μ	1:4	16μ	22μ	1:1,3	9μ

DISCUSSION ET CONCLUSIONS

Tout en étant connues depuis plus de 150 ans (9), les Grégarines parasites des Crustacés Amphipodes comptent actuellement environ 25 espèces présentes chez des genres d'eau douce et marins, tels *Gammarus, Orchestia, Talitrus, Caprella, Pontoporeia, Pallasea, Ampelisca, Synurella, Pontogammarus* (1)(2)(4)(5)(7)(10). Dès les premières descriptions, deux types de grégarines furent mis en évidence: l'un trapu, volumineux et l'autre très allongé, filiforme. Bien que leur appartenance générique soulève encore des incertitudes, pour des raisons déjà exprimées (1) il nous semble opportun de maintenir pour la forme ramassée le genre *Cephaloidophora* Mavrodiati 1908(8)(10), auquel on rapporte également nos espèces nouvellement décrites, certainement sous la reserve d'une révision à fond du statut systématique des Grégarines des Crustacés, premièrement par la connaissance comparée de la forme des spores.

En établissant les six nouvelles espèces de *Cephaloidophora*, dont les diagnoses sont précisées ci-dessus, on élargit la liste d'Amphipodes parasitées par les Grégarines avec de nouvelles hôtes, genres ponto-caspiens, appartenant aux familles *Gammaridae* et *Corophiidae*, qui, par leur espèces présentes surtout dans le Delta du Danube et dans la région littorale de la Mer Noire, constituent environ 43 % de la faune totale ponto-caspienne connue (3). Des représentants de la fam. *Corophiidae* sont pour la première fois cités comme hôtes des grégarines.

Les formes que nous venons de décrire dans le présent travail, c'est-à-dire: *Cephaloidophora mucronata* n. sp., *C.similis* n. sp., *C.corophii* n. sp., *C.rotunda* n. sp., *C.elongata* n. sp., et *C.parva* n. sp. se montrent plutôt apparentées, par leur forme et les particularités des cycles évolutifs, à des espèces de *Cephaloidophora* connues chez des amphipodes marins (10) tels *C.maculata* Léger et Duboscq 1911 (chez *Gammarus marinus*) et *C.talitri* Mercier 1912 (chez *Talitrus saltator*), qu'à des Caphaloidophora parasites des amphipodes des eaux douces intérieures, tels *C.synurellae* Bălcescu 1972 et *C.margaretae* Bălc. 1972 (chez *Synurella ambulans*) (1) ou *Rotundula gammari* (Diesing 1859) Goodrich 1949(chez *Gammarus pulex* et *G.roeselii*) (5). Il y a là encore une preuve de l'ancienneté et de la specificité du parasitisme des Grégarines, dont l'évolution est étroitement liée à celle de leurs hôtes, en faveur d'une lointaine, mais incontestable origine marine.

Les amphipodes relictes du système ponto-caspien, retirées actuellement dans les eaux douces du Delta du Danube ou les lacs du littoral de la Mer Noire, ont perpetué dans leur milieu secondairement adouci, des Grégarines initialement propres aux hôtes marines.

Cettes nouvelles donées, sur les Cephaloidophores des amphipodes ponto-caspiens de Roumanie, s'ajutent à nos constatations antérieures (2) sur la présence d'une grégarine à syzygies multiples raminfiées, Uradiophora ramosa Bălcescu-Codreanu 1974, le seul cas connu chez une hôte amphipode d'eau douce. Vu que les grégarines de ce type se rencontrent surtout dans certaines familles parasites des Crustacés marins, nous avons également interprété ce maintien de l'aptitude à produire des syzygies multiples comme preuve de l'origine marine de son hôte, *Pontogammarus robustoides* (Grimm. 1894) A. Martinov 1924, une autre espèce d'amphipode ponto-caspien ayant subi l'isolement écologique dans les eaux douces du Delta du Danube.

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Institut de Biologie
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DISTINCTION TAXONOMIQUE ENTRE LES ESPÈCES PALÉARCTIQUES *LIOSARCOPHAGA AEGYPTICA* (SALEM) ET *LIOSARCOPHAGA PARKERI* (ROHDENORF) ET DESCRIPTION DE DEUX ESPÈCES NOUVELLES AFROTROPICALES (*DIPTERA, SARCOPHAGIDAE*)

ANDY Z. LEHRER

The correct taxonomical distinction between the palaearctical species *Liosarcophaga aegyptica* (SALEM, 1935) and *Liosarcophaga parkeri* ROHDENDORF, 1937 is shown. Two new afrotropical species : *Liosarcophaga salemiana* sp. n. from South Africa and *Liosarcophaga sabae* sp. n. from Eritrea are described.

INTRODUCTION

Dans sa monographie sur les Sarcophagines éthiopiennes, ZUMPT (1972:168) a mentionné aussi l'espèce paléarctique «*Sarcophaga aegyptica* SALEM». En réalité, il n'a pu jamais l'identifier, en dépit de ses discussions sur l'historique imaginaire de cette dernière. Cependant, il est significatif de retenir qu'il a introduit *S. inzi* CURRAN (ZUMPT, 1951:177) dans sa synonymie et qu'après l'examen d'un exemplaire mâle, existant dans les collections du British Museum et déterminé par SALEM lui-même, il n'a pas réussi de retenir les caractères spécifiques qui distinguent, au moins, *Liosarcophaga aegyptica* d'une espèce tout à fait différente que nous avons nomée *Liosarcophaga sabae* sp. n.

D'après les renseignements inexacts de ZUMPT, l'espèce *Liosarcophaga aegytica* (SALEM, 1935) a été mentionnée mécaniquement dans la faune de la région éthiopienne par ROHDENDORF (1963 : 9) et puis par DEAR (1980 : 815) et VERVES (1986 :166).

Mais, le point du départ des erreurs d'identification de *Liosarcophaga aegyptica* et ses espèces affines, paléarctiques ou africaines, se trouve dans la conception antitaxonomique et superficielle de GREGOR & POVOLNY (1960:172) qui ont exercé des pressions non-justifiées sur ROHDENDORF afin d'obtenir la reconnaissance que *Parasarcophaga (Liosarcophaga) parkeri* est un synonyme de l' espèce de SALEM. Bien que cette procédure de nomenclature n'eût pas été fondée sur la comparaison concrète des types de ces deux espèces, en fin de compte ROHDENDORF a cédé. L'erreur s'est perpétuée ainsi d'une manière non critique dans les conceptions des autres spécialistes qui ont abordé l'étude des Sarcophagines africaines.

Néanmoins, il faut souligner que ROHDENDORF (1937) a fait une distinction correcte entre *aegyptica* SALEM et sa *parkeri*, en précisant, dans ses clés de détermination des espèces du genre *Parasarcophaga* (s. lat.), une série d'importants caractères de leurs génitalia mâles (la forme des apophyses latérales de

la partie apicale du paraphallus et celle des lobes membranaux internes). D'une manière implicite, SÉGUY (1941:121, fig. 149) l'a admise. Mais, après l'intrusion de GREGOR & POVOLNY (supra), *Sarcophaga aegyptica* est devenue le spectre des illusions de quelques espèces (parmi lesquelles *Liosarcophaga parkeri* aussi).

Dans les collections du Natal Museum, nous avons trouvé une espèce-jumelle sud-africaine de *Liosarcophaga aegyptica* (SALEM, 1935), que nous avons baptisée *Liosarcophaga salemiana* sp. n. et qui nous a donné la possibilité de constater le degré de parenté tant entre celles-ci qu'entre elles et *Liosarcophaga parkeri* ou *Liosarcophaga sabae* sp. n. Les différences en ont été facilement établies par nous, parce qu'en Roumanie *Liosarcophaga parkeri* est assez répandue et, dans nos collections, elle est bien représentée au point de vue numérique.

DESCRIPTION DES ESPÈCES

1. Liosarcophaga aegyptica (SALEM,1935)

= *Sarcophaga dux var. aegyptica* SALEM 1935, Egypt. Univ., Fac. Méd., Publ. 5:56.
= *Parasarcophaga (Liosarcophaga aegyptica)* sensu ROHDENDORF 1937, Faune de l'URSS, Insectes Diptères,19 (1):220; - sensu VERVES 1986 (partim), Catalogue of palaearctic Diptera, **12**:166 - **syn. n.**
= *Sarcophaga misera* var. *aegyptica* sensu SÉGUY 1941, Encycl. Ent., A **21**:121, fig. 149 - **syn. n.**

2. Liosarcophaga parkeri ROHDENDORF,1937

=*Parasarcophaga (Liosarcophaga) parkeri* ROHDENDORF 1937, Fauna de l'URSS, Insectes Diptères, **19** (1):217.
= *Parasarcophaga (Liosarcophaga) aegyptica* sensu GREGOR & POVOLNY 1960, Čas. Česh. společ. ent., **57**(2):172, tab. 3,
f ig. 1. - sensu FAN Z I -DE 1965, Key of the common synanthropic flies in China, 270, fig. 1036-1037. - sensu VERVES 1986 (partim), Catalogue of palaearctic Diptera **12**:166 - **syn. n.**
=*Parasarcophaga aegyptica* sensu ROHDENDORF 1970, Opred. Nasek. Evrop. Časti SSSR. 5(2):660, fig. 860, 4-5. - sensu MIHALYI 1979, Fauna Hung. 135, **16**:129 - **syn. n.**

3. Liosarcophaga salemiana sp. n.

MÂLE

Tête. Noire et couverte d'un tomentum argenté de front, vu du dessus et au lieu le plus étroit, mesure 1/2 de la largeur d'un œil. La bande frontale est deux fois plus large qu'une parafrontalie. Antennes noires à légère teinte brunâtre sur les articles basaux; le troisième article est presque deux fois plus long que le deuxième.L'arista brune noirâtre a des poils moyens sur les deux parties. Trompe et palpes noirs de péristome mesure 1/3 du grand diamètre oculaire.

Chétotaxie de la tête. Macrochètes verticaux internes longs, forts et rétroclines;les macrochètes verticaux externes manquent; ocellaires proclines et préverticaux rétroclines bien développés, mais les premiers sont un peu plus minces et plus courts;les macrochètes frontaux sont au nombre de 10-11 paires; macrochètes parafaciaux minces et assez courts; les petites vibrisses montent sur 2/3 des bordures faciales; les microchèts occipitaux sont disposés sur un rang. Péristome couvert de poils noirs; la partie postérieure de la tête est pourvue de poils blancs jaunâtre.

Thorax. Noir, avec tomentum argenté, trois bandes médio-dorsales longitudinales noires larges et deux bande latérales étroites. Propleures glabres; prosternum poilu. Stigmates bruns noirâtre. Les pattes sont noires à teinte légère sur les tibias; les fémurs médians ont un ctenidium typique.

Chétotaxie du thorax. ac =0 + 1, dc = 5 + 4 - 5, ia = 1 + 3, prs = l, sa = 3, h = 3, ph = 2, pa = 2, sc = 3-4 + l, pp = 1 (plus quelques poils), pst = l, st = l : l : l.

Ailes. Transparentes et légèrement brunâtres. Epaulette noire; basicosta et costagium jaunes. La nervure r_1 glabre; la nervure r_{4+5} pourvue de cils jusqu'à la moitié de la distance entre son origine et r-m.Le cubitulus est courbé en angle droit et prolongé d'un pli. Épine costale petite. Écailles blanches; balanciers brunâtres et par endroits d'un brun foncé.

Chétotaxie des tibias. Les tibias antérieurs ont 2-3 ad proximaux et 1 pv; les tibias médians pourvus de 2 ad, 1 av, l pd médian, 2 pd proximaux courts et 1 pv; tibias postérieurs ont 2 ad, 1 av, 2 pd et une longue pilosité sur les parties antéro- et postéroventrales.

Abdomen. Noir, avec le tomentum argenté et dessins en damiers. La formule chétotaxique: 0 + 0 (2 couchés et piliformes) + 2 + série. Tergite génitale noir, avec un peu de tomentum et sans macrochètes marginaux. Tergite anal brun noirâtre.

Armature génitale: fig. l. Elle est très semblable avec celle de L. parkeri ROHDENDORF de Roumanie (f ig. 2). Le sternite V (A) n'a pas de brosses, mais tant la partie basale, que les lames latérales sont plus larges, les dernières étant pourvues de 2 macrochètes longs à leurs bouts. Cerques (B) de forme rapprochée; mais, la partie apicale est étroite et ondulée vers son sommet, se courbant un peu latéralement; les paralobes sont plus ou moins triangulaires, leurs angles étant arrondis. Distiphallus (C) de longueur moyenne. Partie basale du distiphallus plus ou moins longtriangulaire et, vue de profil, est prolongée de lobes paraphalliques très étroits et peu courbés. La partie apicale du paraphallus est celle qui présente les caractéristiques spécifiques les plus importantes pour sa distinction de l'espèce affine. Elle a une pièce médiane relativement très courte, triangulaire et avec un sommet légèrement courbé; ses apophyses latérales sont longs, très larges (vues apicalement), ployées latéralement en angle droit (comme un fer cornier) et très élargies à leur partie terminale. Membrana pliée et pigmentée. Lobes membranaux courts; ceux internes sont très sclérifiés, courts et bidentés; ceux externes étroits et transparents. Les styles sont relativement courts et n'arrivent pas jusqu'au bout des apophyses latérales. Prégonites (D) et postgonites (E) sont presque égaux; les premiers sont étroits, droits et courbés au sommet; les seconds ont une forme de crochet, étroits, avec leur base très peu élargie et 2 macrochètes médians sur leur marge superieure.

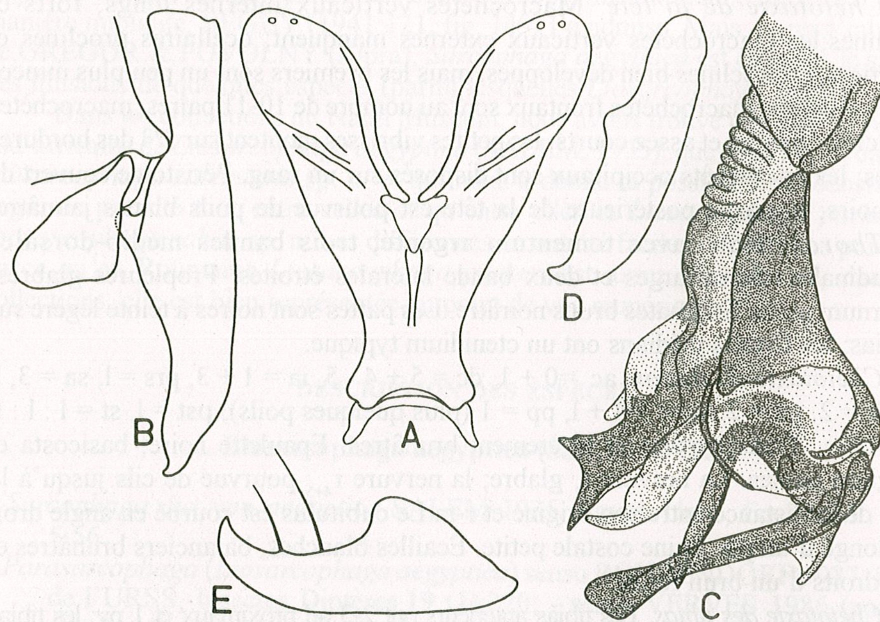

Fig.1.-Armature génitale mâle de *Liosarcophaga salemiana* **sp. n.** -A: sternite V; B: cerques et paralobes; C: distiphallus; D: prégonites; E: postgonites

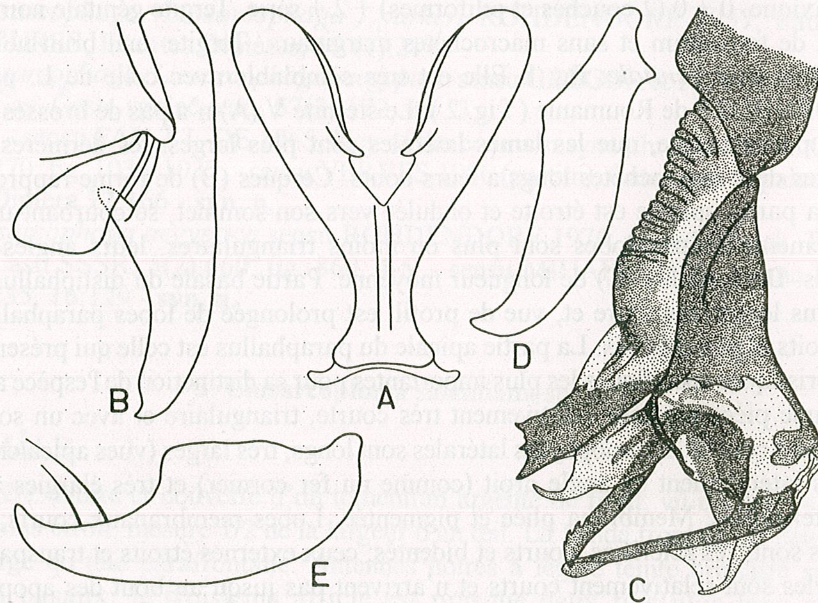

Fig. 2. - Armature génitale mâle de *Liosarcophaga parkeri* ROHDENDORF. -A: sternite V; B: cerques et paralobes; C: distiphallus; D: prégonites; E: postgonites

Longueur du corps: 9,5 - 10,5 mm.

FEMELLE. Inconnue.

Matériel-type. Holotype 1 ♂ et 1 ♀ paratype avec l'étiquette suivante: "SOUTH AFRICA: Natal, Drakensberg Garden Area, 2929 CA, Date: 13 - 14.X.1984, Coll. J.G.H.Londt".

4. **Liosarcophaga sabae** sp. n.

= *Sarcophaga aegyptica* sensu ZUMPT 1972 (nec SALEM 1935), Explor. Parc natn. Virunga. Miss. G.F de Witte, Bruxelles, **101**:168-169, fig. 97 - **syn. n.**
= *Parasarcophaga (Liosarcophaga) aegyptica* sensu VERVES 1986 (partim),

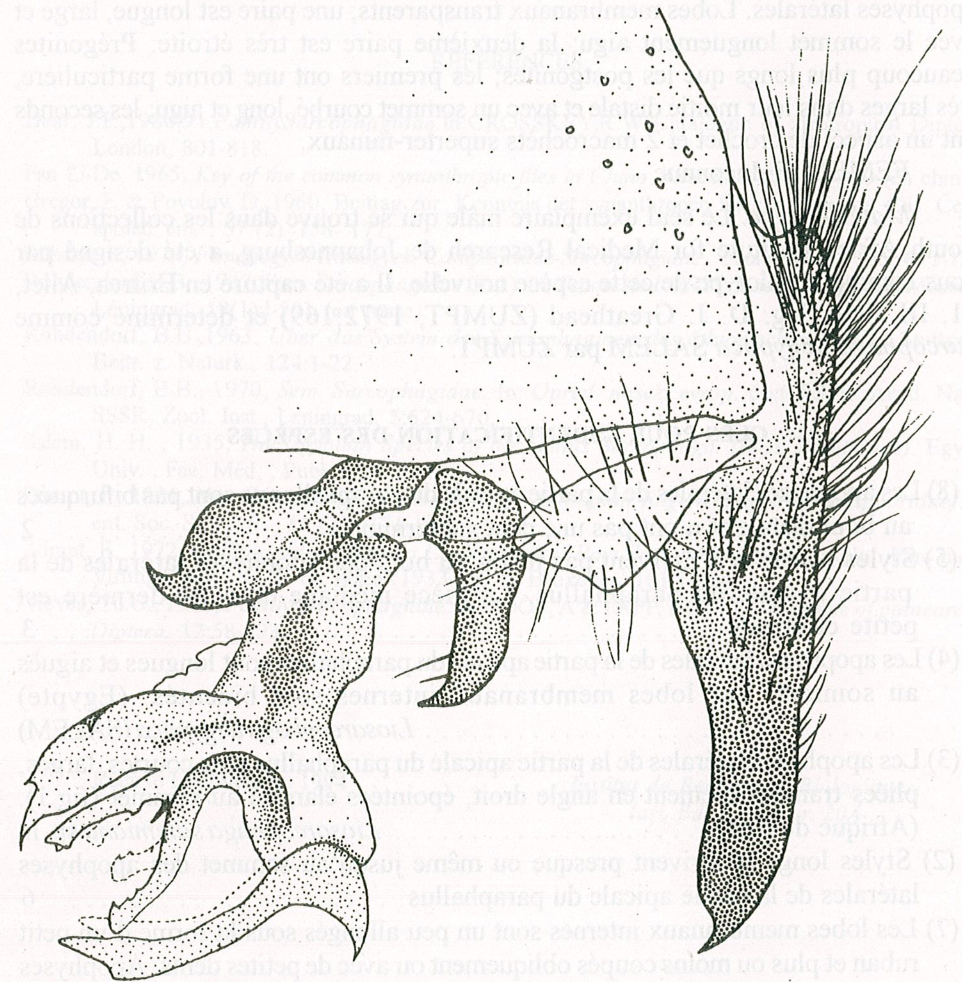

Fig. 3. - Armature génitale de *Liosarcophaga sabae* **sp. n.** - Selon ZUMPT 1972 : 169, fig. 98 (un peu modifié).

Catalogue of palaearctic Diptera, **12** :166 - **syn.n.**

MÂLE

Armature génitale: fig. 3 (selon ZUMPT 1972:169, fig. 97, un peu modifiée). Les cerques sont élargis dans leur zone médiane, puis s'amincissent et forment apicalement un sommet allongé et aigu; les paralobes n'ont pas une forme particulière.Le distiphallus est très caractéristique. La partie basale du paraphallus est très allongée, plus ou moins rectangulaire et puis s'étrangle beaucoup (? artéfacte) au niveau des lobes membranaux; les lobes paraphalliques ne sont pas figurés. La partie apicale a une pièce médiane allongée et aiguë; les apophyses latérales sont très larges et terminées dans un sommet long, plus ou moins aigu et un peu plus étroit que la largeur des apophyses. Styles longs et arrivent presqu'au bout des apophyses latérales. Lobes membranaux transparents; une paire est longue, large et avec le sommet longuement aigu; la deuxième paire est très étroite. Prégonites beaucoup plus longs que les postgonites; les premiers ont une forme particulière, très larges dans leur moitié distale et avec un sommet courbé, long et aigu; les seconds ont un aspect de crochet et 2 macrochèts superter-minaux.

FEMELLE. Inconnue

Matériel-type. Le seul exemplaire mâle qui se trouve dans les collections de South Africa Institute for Medical Research de Johannesburg, a été désigné par nous comme le holotype de cette espèce nouvelle. Il a été capturé en Eritrea, Ailet, 11. III.1956, leg. D. J. Greathead (ZUMPT, 1972:169) et déterminé comme *Sarcophaga aegyptica* SALEM par ZUMPT.

CLÉS POUR L'IDENTIFICATION DES ESPÈCES

1 (8) Les apophyses latérales de la partie apicale du paraphallus ne sont pas bifurquées au bout distale et n'ont pas une dent subterminale 2

2 (5) Styles courts et n'arrivent pas jusqu'au bout des apophyses latérales de la partie apicale du paraphallus. La pièce médiane de cette dernière est petite et étroite 3

3 (4) Les apophyses latérales de la partie apicale du paraphallus sont longues et aiguës au sommet. Les lobes membranaux internes sont bidentés. (Egypte) . . . . *Liosarcophaga aegyptica* (SALEM)

4 (3) Les apophyses latérales de la partie apicale du paraphallus plus courtes, larges, pliées transversalement en angle droit, épointées élargies au sommet (fig.1). (Afrique du Sud) . . . . *Liosarcophaga salemiana* sp. n.

5 (2) Styles longs et arrivent presque ou même jusqu'au sommet des apophyses latérales de la partie apicale du paraphallus 6

6 (7) Les lobes membranaux internes sont un peu allongés sous la forme d'un petit ruban et plus ou moins coupés obliquement ou avec de petites dents. Apophyses latérales de la partie apicale du paraphallus sont étroites et arrondies au bout. La pièce médiane de la partie apicale est plus développée et plus large. Les prégonites sont relativement étroits et ont le sommet légèrement courbé. Les postgonites ont la base très large (fig. 2). (Europe orientale, Asie occidentale) . . . . *Liosarcophaga parkeri* ROHDENDORF

7 (6) Les lobes membranaux internes ont un aspect plus ou moins foliacé et aigus au bout. Les apophyses latérales de la partie apicale du paraphallus sont larges, légèrement courbées, brusquement étroites à leur partie subterminale et plus ou moins aiguës au bout. La piéce médiane apicale est longue, étroite et aiguë.Les prégonites sont très longs et très dilatés à la partie submédiane. Les postgonites sont courbés aux parties basale et apicale (fig. 3). (Eritrea) . . . . *Liosarcophaga sabae* sp. n.

8 (1) Les apophyses latérales de la partie apicale du paraphallus sont bifurquées au bout ou sont pourvues d'une dent subterminale 0

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MICROTETRAMERS SP. LARVAE (NEMATODA : TROPISURIDAE) ENCYSTED IN THE STOMACH WALL OF TOAD, *BUFO VIRIDIS* IN IRAQ

ZOHAIR I. F. RAHEMO, SULAIMAN N. AMI

INTRODUCTION

Microtetrameres sp. larvae (Nematoda : Tropisuridae) have been found for the first time in Iraq encysted in the stomach wall of the green toads, *Bufo viridis*. Due to the lack of genitalia it was not possible to identify these larvae up to the species level. Histological investigations of this cyst revealed that single larva encysted in each cyst. Proliferation of cells was accompanied by each encystement mainly fibroblast. The route of penetration of these larvae in the stomach wall was also observed.

Scarce information is available on parasites of Iraqi amphibians. The first report recorded two species of flukes in frogs of southern region of Iraq (Saoud and Roshdy, 1970). Al - Barwari and Nassir (1983) recorded five species of amphibian parasites including four species of nematodes, namely, *Aplectana* sp., *Cosmocerca* sp. *Oswaldocruzia filiformis* and *Microtetramers* sp. In the northern region of Iraq, the first studies were carried out on protozoan and trematode parasites of amphibians of Neinava district (Dauood, 1975). Other reports on the amphibians of northern Iraq were mainly surveying the trematode parasites of Arbil province (Saeed *et al.*, 1992), and on haematozoons (Molan *et al.*, 1989). This finding comes as a result of general amphibian investigations which record and describe for the first time larval nematods encysted in the wall of toad stomach, *Bufo viridis*, in Iraq.

MATERIALS AND METHODS

Two hundred toads, *Bufo viridis* Laurenti, 1798, were dissected out during 1991-1992. Only 1 female was infected with nematodes encysted in the wall of *Bufo viridis*. Some of these cysts were ruptured, nematode specimens mounted in glycerine in order to study their general morphology. Some cysts with stomach wall were fixed in Bouin fluid, embedded in paraffin wax, sectioned at 8-10 μm in thickness, stained in haematoxylin - eosin. Drawings of these nematodes were made using Camera lucida. Photomicrographs of sections were taken by the Olympus photomicrograpic system 10 AD.

REV. ROUM. BIOL.– BIOL.ANIM., TOME 40, N° 1, P. 19–23, BUCAREST, 1995

RESULTS

More than 15 cysts or nodules were observed in the stomach of this infected toad, *Bufo viridis*. These cysts are protruded from the outer layer of the stomach measuring 2-4 mm in diameter. The nematode specimens obtained from the cysts of the stomach were at larval stage and each cyst contained one larva.

The total body length is 2.5-2.9 mm and the width is 0.2-0.3 mm. Three small lips are present surrounding the buccal capsule leading into the pharynx which is muscular then becomes glandular. The oesophagus leads into the intestine which ends into an anus at the posterior of the body. The anal opening is 0.7-0.8 mm from the posterior extremity of the body. The tail is pointed (Fig. 1).

Histological examination of these cysts revealed that these larvae are encysted in the serosa or in the outer layer of the stomach wall (Figs. 2-4) forming an isolated region of nodule outside the stomach clearly seen after general dissection. In a section a clear route was observed in which the larva appears to penetrate the wall and migrates to the outer surface of the stomach (Figs. 5,6). Proliferation of cells especially fibroblasts appears to be accompanied by such a penetration (Fig. 7). No sign of calcification around the nematodes was observed.

DISCUSSION

If the characters of these larvae are compared to the characters listed by the key of Yamaguti (1961) these larvae resemble *Microtetramers* sp. especially in the presence of the barrel-shaped buccal capsule, the presence of the three small lips surrounding the buccal capsule. Species identification of such larvae is impossible due to the absence of the adult male and female characters. Al - Barwari and Nassir (1983) reported this parasite from the stomach and intestine of *Bufo viridis* and thought that it is an accidental parasite especially because it is found in an unusual site, i.e. stomach of the host. It is worth mentioning that Al - Barwri and Nassir (1983) found that the adult stage of female only and they gave the classification at the level of genus due to the absence of male specimens. Yamaguti (1961) stated that males of Tropisuridae live free in the intestinal lumen while females are encysted in the proventriculi of birds. Therefore, it is possible that these nematodes which are encysted in the stomach of *Bufo viridis* may develop after moulting into the adult stage and then escape into the stomach or intestinal lumen. Al - Barwari and Nassir (1983) stated that these nematodes come after the digestion of infected insects, viz., grasshoppers or beetles. However, in one specimen, penetration of the stomach wall was observed (Fig. 7), which verifies the speculations of Al - Barwari and Nassir (1983). Also it is concluded that this nematode is an accidental parasite as it is found only in 0.5% of the examined toads.

The cyst walls surrounding these larvae are not similar to those cysts surrounding the larvae of *Trichinella spiralis* (Solusby, 1969) encysted in the human or murine muscles as there is no sign of calcification. However, it is

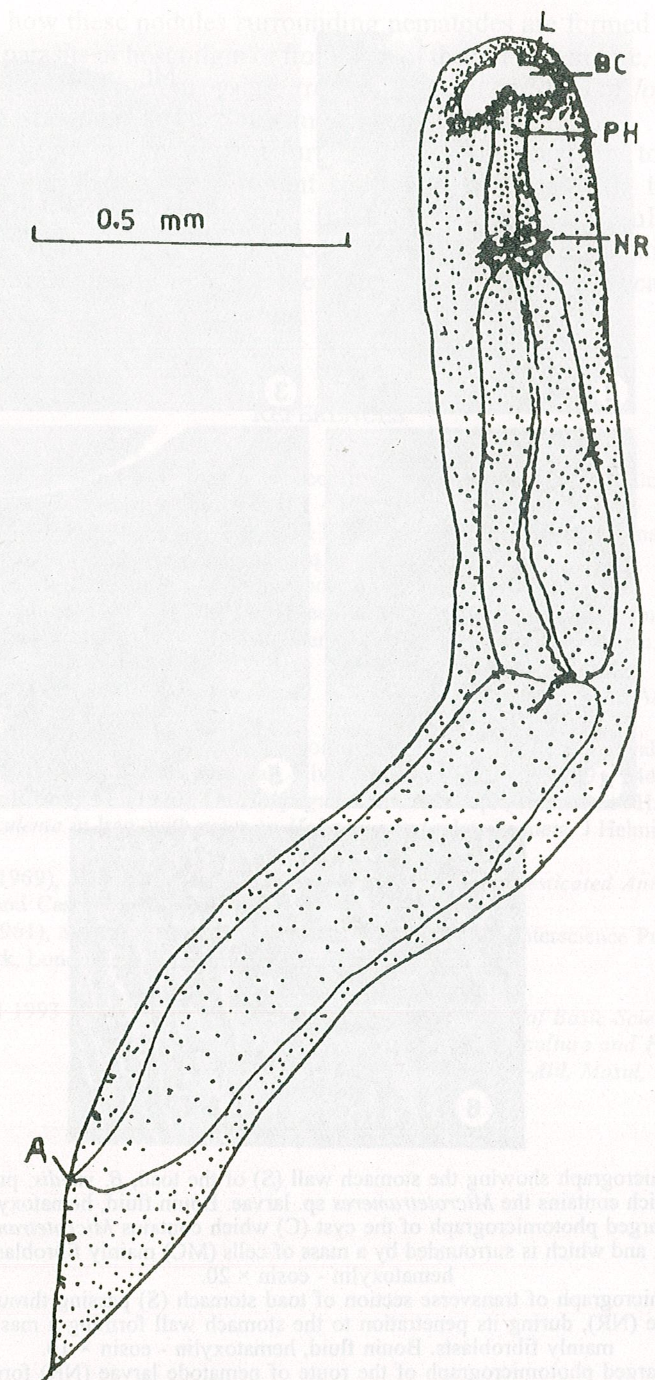

Fig. 1.- A camera lucida drawing of the *Miceoletrameres* sp. larvae showing the buccal capsule (BC), surrounded by three small lips (L), pharynx (PH), nerve ring (NR), and the anal opening (A).

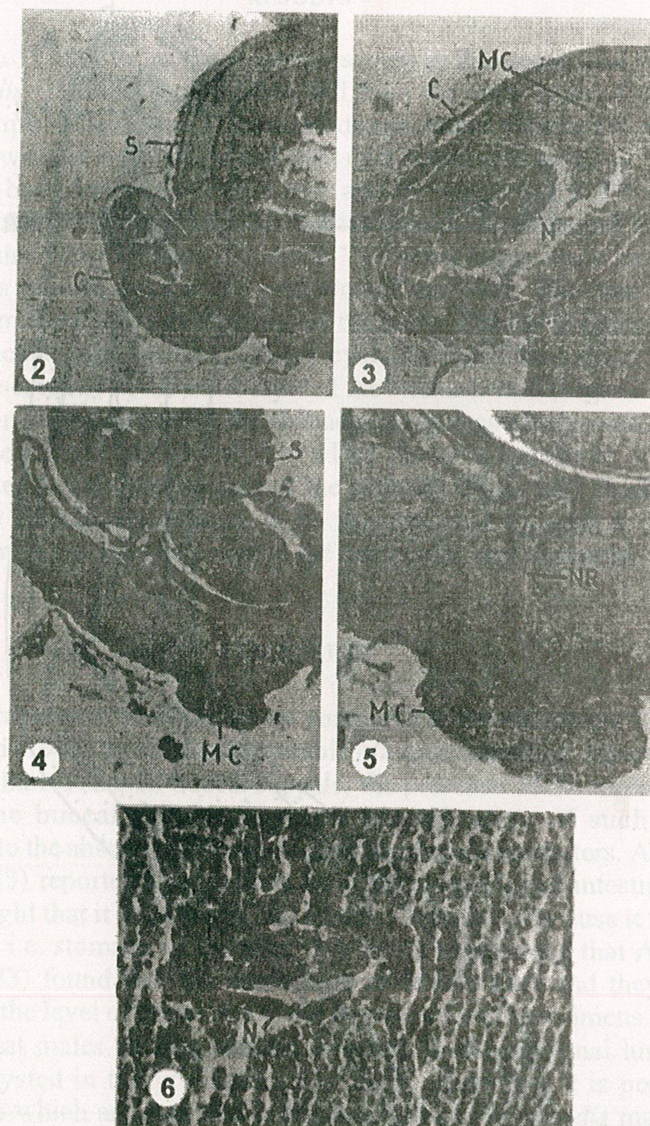

Fig. 2.- Photomicrograph showing the stomach wall (S) of the toad, *B. viridis*, protruded from it the cyst (C) which contains the *Microtetrameres* sp. larvae. Bouin fluid, hematoxyllin-eosin × 10.
Fig. 3.- An enlarged photomicrograph of the cyst (C) which contains *Microtetrameres* sp. nematode larvae (N), and which is surrounded by a mass of cells (MC) mainly fibroblasts. Bouin fluid. hematoxylin - eosin × 20.
Fig. 4.- Photomicrograph of transverse section of toad stomach (S) passing through the route of nematode larvae (NR), during its penetration to the stomach wall forming a mass of cells (MC) mainly fibroblasts. Bouin fluid, hematoxylin - eosin × 10.
Fig. 5.- An enlarged photomicrograph of the route of nematode larvae (NR) forming a mass of cells (MC). Bouin fluid, hematoxylin - eosin × 20.
Fig. 6.- An enlarged photomicrograph showing the *Microtetramere* nematode larvae (N) embedded in the mass of cells mainly fibroblasts Bouin fluid, hematoxylin - eosin × 40.

questionable how these nodules surrounding nematodes are formed whether they derived from parasits or host origin or from both of them. Furthermore, no distinction of layers as those observed in cysts surrounding larval cestodes of *Joyuexiella*. sp. (Rahemo and Sulaiman, 1989; Salih and Rahemo, 1988).

It is suggested therefore that further studies are necessary to identify the larvae which may belong to different species possibly specific for toads and consequently different from those infecting the proventriculus of birds. Experimental studies appear much needed to reveal more information about the route of larvae during infection, and the histopathological changes caused by such nematode larvae.

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ELECTRON MICROSCOPICAL STUDY OF METHANOGENE BACTERIA ISOLATED FROM TECHIRGHIOL LAKE

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DC_1, bacterial population isolated from the Techirghiol Lake mud, at ultrastructural level, reveals a natural association of bacteria in which methanogene species of *Methanogenium* and *Methanobacterium* genera are predominant. The cells ascribed to *Methanogenium* genus have a cell wall that structurally is similar to that of Gram positive bacteria, but much thinner.
The growth on LPBM nutritive medium, without adding any NaCl, causes the emergency of some cells having unusual shapes of *Methanogenium* genus. Their presence represents a consequence both of the low tonus of growth medium as compared to the natural medium from where they were isolated and of the elasticity of cell wall. The images suggest the division development by strangling and in aberrant forms, by multiple fragmentation of the cell.
Methanobacterium genus cells maintain their characteristic appearance, indicating the rigidity of their cell wall.

Methanogene bacteria have a unique particularity among prokaryote microorganisms: they produce methane as a major result of anaerobic metabolism. This physiologic feature has been proposed in 1956 by Barker as a metabolic defining feature of a group of bacteria very diverse morphologically that he named *Methanobacteriaceae* (2).

The taxonomy of this physiologically well defined group was rendered difficult by the difficulties to isolate methanogene bacteria in pure cultures. The various species were named on the basis of the energetic substratum converted to methane by the so-called "purified" cultures which in most cases have been proved to be an association of methanogene and nonmethanogene bacteria.

All methanogene bacteria are strictly anaerobic and reduce CO_2 with H_2. This metabolic process results in methane. Some bacteria make methane from simple organic substrata: formiate, methanol, acetate, methylamine. By their physiologic interaction with chemical heterotrophic bacteria, via reactions of H_2 transfer, methanogene bacteria perform an important ecologic function in the natural habitats. They mediate the terminal steps of the anaerobic cycle of organic material to methane and CO_2.

Using the molecular criteria (nucleotide sequence in RNA 16 S, chemical composition analysis of the cell wall) has lead to a major taxonomic revision of methanogene bacteria (1). The methanogene bacteria are widespread in nature: in anaerobic, aquatic environments, in deposits in which the organic material is decomposed, in the animal intestinal tract, in digesters for residual waters. They also have been found in tree wood in thermal springs, in the depth of oceans.

Few microorganism groups have such a morphologic diversity as methanogene bacteria. The following major morphologic types are distinct: cocci, bacilli, spirilla, sarcina. The methanogene bacteria exhibit significant variations as concerns the build - up and chemical composition of a certain cell structure, primarily of the cell wall. Only *Methanobacterium* has a wall with the typical structure of positive gram bacteria. The other representatives have the cell wall built according to a model (pattern) similar to that of positive gram bacteria but it is thinner.

Ultrastructural investigations have been performed with *Methanospirillum hungatii* (10), *Methanosarcina* (7,8,11), *Methanobacterium* (3,10), etc.

In the samples harvested from the Black Sea, where anaerobic conditions are predominant, Romesser et al. (1979) have described *Methanogenium* genus (6).

In the following there are presented some aspects of the fine structure of methanogene isolated from the Techirghiol Lake mud.

MATERIAL AND METHODS

The culture from the collection of Microbiology Laboratory (DC_1 population isolated from the Techirghiol Lake mud) grown on a LPMB medium (10) were centrifuged at 2500 rpm. The cellular deposit had undergone a double fixation with 2% glutaraldehyde and 1% osmium tetraoxide in 0.2 M phosphate buffer, pH 7.2.

Afterwards the samples were processed according to the classical method of electron microscopy technique. The fine sections made under Tesla ultramicroton, contracted with uranyl acetate and lead citrate, were examined under a Philips 201 electron microscope.

RESULTS

In DC_1 population isolated from Techirghiol Lake methanogene bacteria are predominant. Those associated, the nonmethanogene ones, are especially sporulated.

Morphologically, the methanogene bacteria of DC_1 population may be assigned to *Methanogenium* genus, with 2 species: *M. marisnigri* and *M. cariaci*. In an inferior numeric ratio there are the cells of *Methanobacterium sp.*

Bacterial cells assigned to *Methanogenium* genus have an extremely diverse morphology. They have an amoebal appearance with sizes ranging from 0.5 at 3 μm (Fig.1). The form diversity is correlated with the lack of rigidity of the cell wall. At the ultrastructural level their cell wall is clearly distinct from that of negative Gram bacteria (4,5), by the absence of external membrane. In most methanogene bacteria, the wall is thin and compact. Chemically, the muramic acid is absent, one of mureine components conferring rigidity and strength to the positive Gram bacteria wall.

Cytoplasmic membrane is compact, frequently electron dense, with a high ribosomal content.

The nuclear material has a compact distribution, frequently ring shaped, in the central zone of the cell (Figs.2,3) or seldom, dispersed into the cytoplasm.

Nucleoid morphology probably reflects different stages of the cell cycle: the young ones have a compact nuclear material, with well individualized fibrils (Fig. 3), whereas in the aged ones the nucleoid is dispersed into electron clear zones without precise limits (Fig.4). Frequently bacilli appear with a bar bells aspect (Fig.5) or distorted (Fig.6). Probably, these cells belong to *M. marisnigri* species.

Sometimes the cells of *Methanogenium* genus have as a major morphological feature a reduced electron density of the cytoplasm, the nuclear material dispersed and the cell wall is thinner (Fig.7) or "eaten by moths" on extensive areas (Fig.8). Very frequently there appear cells with aberrant morphology: Y,V,L,T, with smaller sizes than those above described, reminding the *Rhizobium* sp. bacteroids from leguminous plants, nodosities (Fig.1).

No internal membranous structure of mesosome type was signated in the cells assigned to *Methanogenium* genus. Seldom, vacuoles of gaseous type (Fig.9) are noticed and are characteristic to *Methanosarcina* (Fig.10).

In the studied population, in a small proportion, bacillar cells are present, belonging to *Methanobacterium* genus (Fig.10).

Fig. 1-9.- ELECTRON MICROSCOPICAL IMAGES OF *METHANOGENIUM* GENUS CELLS.

Fig. 1. – Overall image of association of DC_1 population of methanogene cells. The forms of distorted bacilli Y,T,×38.700 are predominant.

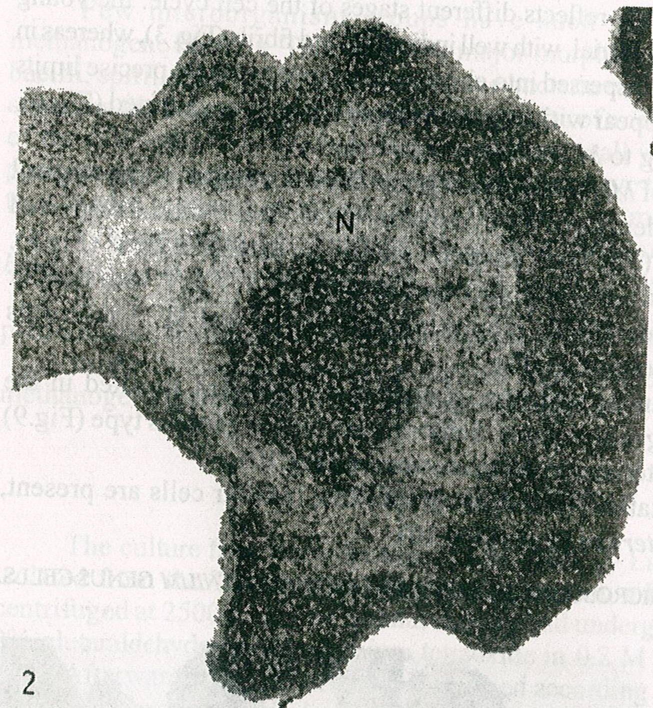


Fig. 2. – *Methanogenium* sp. young cells .The nuclear material (N), of ring shape, is centrally exposed. The cytoplasmis electron dense.

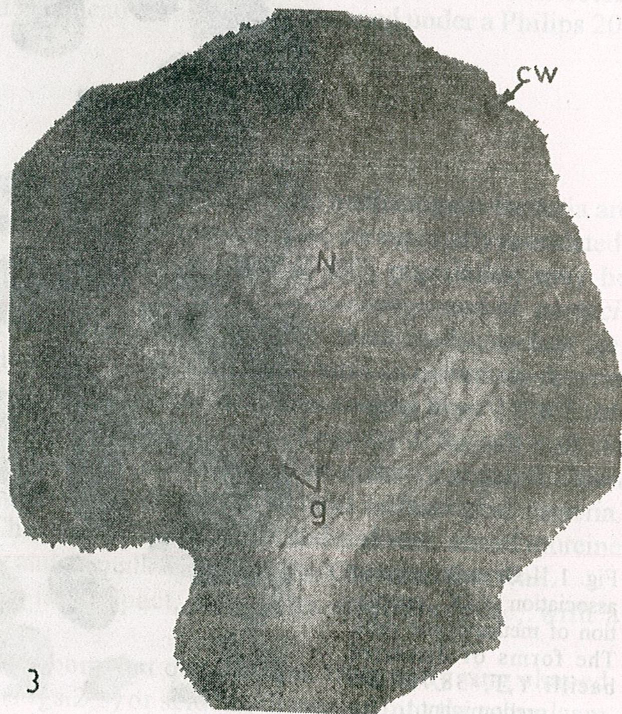
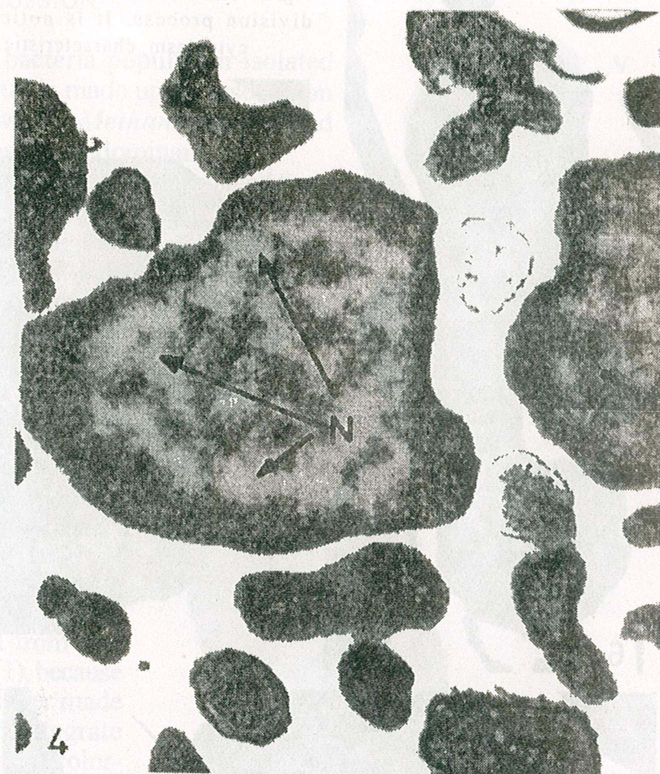


Fig. 3. – The genetic material fibrils are clearly distinct. The cell wall (CW) has a compact structure(g = glycogen inclusions) × 88.800.

Fig. 4.- In aged cells, the nuclear material is dispersed and probably occupies a part of clear electron beaches. The ribosomal charge appears much diminished × 33.000.



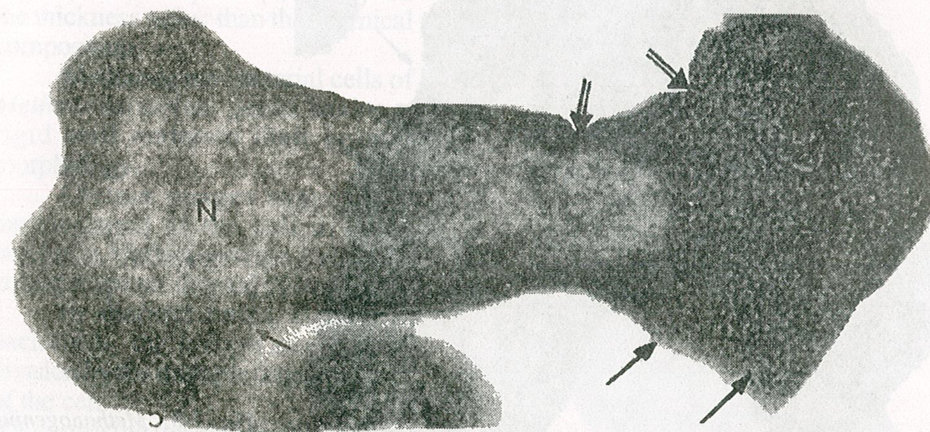


Fig. 5.- Young cell (*Methanogenium sp.*) with bar bells appearance. The cell wall loses its individuality (single arrows). Cell deformation may not be accounted for the maceration of the wall in some areas since in the formation of extreme dilatations participate surfaces with an intact cell wall as well (double arrows) × 88.800.

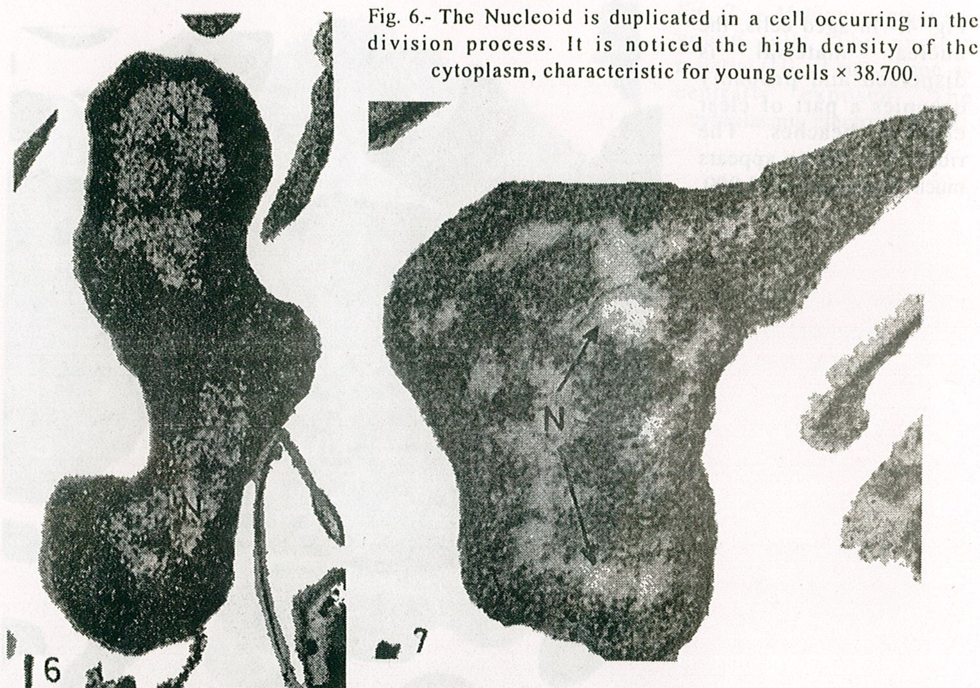


Fig. 6.- The Nucleoid is duplicated in a cell occurring in the division process. It is noticed the high density of the cytoplasm, characteristic for young cells × 38.700.

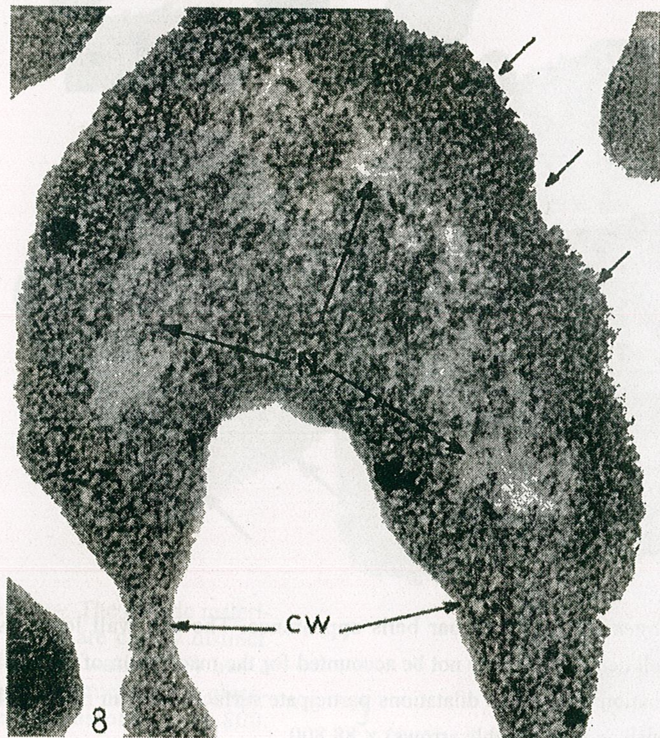


Fig. 7-8.- *Methanogenium sp.* (possibly *M. cariaci*). The cell wall is very thin. On large areas (Fig. 8- arrows) it appears "eaten by moths", but the cytoplasm disintegration is not obvious × 58.800 and 88.800 respectively.

DISCUSSION

DC_1 methanogene bacteria population isolated from Techirghiol Lake mud is made up an association of microorganisms in which *Methanogenium* and *Methanobacterium* species are predominant.

Methanogenium genus bacteria were isolated from the Black Sea (6) and require an additional NaCl in the culture medium. DC_1 population was isolated from a lower salinity than that of the sea. They were grown in LPMB medium without additional NaCl. The lack of medium osmotic pressure as well as the cell wall elasticity are the causes giving rise to aberrant cell forms. There is the possibility that the aberrant forms represent the final stages of development into a lysis of methanogene bacteria. In methanogene bacteria with a coccoid morphology, Gram-negative the rigid bag of the cell wall was not isolated (4,5). At Gram colouring, *Methanogenium* cells give a negative reaction, although their wall is basically different from that of Gram-negative bacteria (1), because it has an external protein layer made of subunits that easily disintegrate (10). The reaction upon Gram coloring seems to depend primarily on the thickness rather than the chemical composition.

Methanogene bacterial cells of *Methanobacterium* genus having a rigid wall maintain their normal morphology.

Certain images suggest the development of the cell division process in *Methanogenium*. Cells with cocoid shape are divided by strangling.

In aberrant forms, similar to bacteroids, the images suggest the division by the multiple fragmentation of the cell.

It is surprising the obvious likeness of morphology of methanogene cells in DC_1 population, with bacteroids in leguminous plant nodosities the convergent morphological evolution being due to the lack of rigidity of the cell wall.

Fig. 9

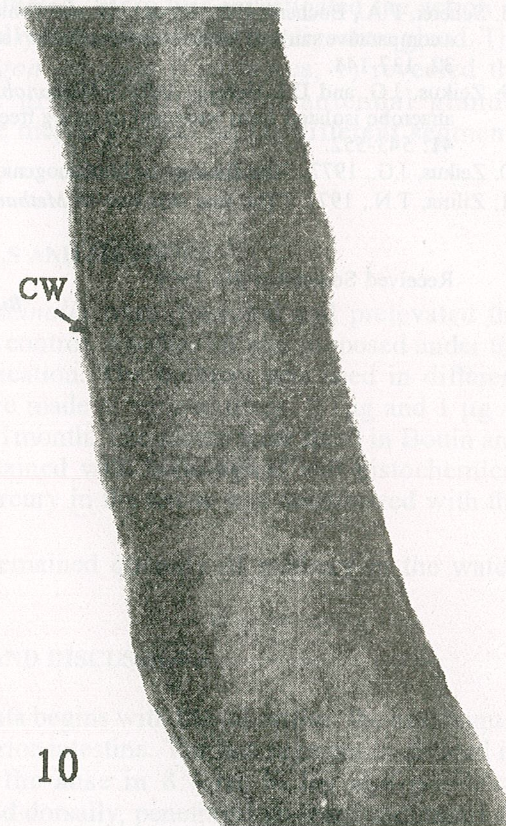


Fig. 10.- Fragment from a *Methanobacterium sp.* cell. The cell wall is thick as compared to that of *Methanogenium* genus cells × 139.200.

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THE ACTION OF MERCURY ON THE DIGESTIVE TRACT AT *ANODONTA CYGNEA PISCINALIS* (LAMELLIBRANCHIATA=BIVALVIA)

MARIA NĂSTĂSESCU, VIORICA MANOLACHE, OTILIA ZĂRNESCU, CĂLIN TESIO

Digestive tract was investigated in optic microscopy at *Anodonta cygnea piscinalis* both in normal and intoxication conditions with mercury chloride. The intoxication doses used were: 0.1 μg and 1μg $HgCl_2$/1/4 months, 5μg $HgCl_2$/1/4 days; 5μg and 10 μg $HgCl_2$/1/1 month. We observed modifications in all segments of digestive tract, both of the epithelial cells and of the muscular, connective and sanguine cells from the other layers.

The investigations about different species of Lamellibranchiata=Bivalvia demonstrate that these animals accumulate and tolerate the big quantity of heavy metals: Hg, Cd, Zn, Pb, etc.(4).

The mercury effect was little studied. More was investigated the action of cadmium (6) which destroyed especially the epithelium of the digestive gland. The electronic microscopy about *Crangon crangon* (Crustaceea, 1) revealed the accumulation of mercury in the gills, digestive and antennal glands.

In this papeer we described the mercury effect on the different segments of the digestive tract.

MATERIALS AND METHODS

A lot of *Anodonta cygnea piscinalis* were dissected and prelevated the segments of digestive system used as control. Another lot was supposed under the influence of mercury chloride intoxication. The polluant was used in different concentrations and sacrifications were made at various times: 0.1μg and 1 μg /4 months; 5μg/4 days; 5μg and 10 μg /1month. The pieces were fixed in Bouin and calcic formol. The sections were stained with histological and histochemical current technics. The presence of mercury in the tissue was emphasized with the autometalographic method (3).

Also, it was appreciated the remained quantity of mercury in the water.

RESULTS AND DISCUSSIONS

The digestive system of Anodonta begins with buccal orifice, the esophagus, stomach (middle intestine) and posterior intestine. The last segment is divided in the anterior part which described the anse in 8 form in leg and posterior part(=rectum). This portion is dirigued dorsally, penetrated pericard and ventricle and open by anal orifice in paleal cavity (5).

Generally, we remark the same histological structure in different segments of the digestive tract. The structural unity we observed especially at digestive tract epithelium which is unistratified, sometimes with different cells.

A basal membrane, connective and muscular tissue exists at the basis of the digestive epithelium.

The epithelium of esophagus has cells of different size with numerous cillia at the apical pole. Some of the cells are prismatic and others, especially the mucocites are of funnel form. The nuclei are situated at different levels.

Also, we observe the phagocytes between the epithelial cells.

The stomach is of ovoide form with thin walls, in which open the diverticles of the digestive gland (hepatopancreas). Also, within the stomach there exists a cecum of crystalline stylet.

The stomach epithelium is unistratified with ciliated prismatic and mucous cells. Under the epithelium there is the connective and muscular tissue. In the connective tissue there are presented numerous amoebocytes.

Also, the intestine has both the prismatic ciliated cells with ovoidal and central nuclei and short cells with spherical nuclei at the base. The last cells have a vacuolar cytoplasm. Between the cells we find the amoebocytes with small granules. A membrane basal positive PAS is at the base of the epithelium.

In the connective tissue we remark the sanguine cells. Some of these have a spherical nucleus with acidophile granulations in the cytoplasm; others with a discoidal peripheral nucleus.

We find the last also between epithelial cells.

The influence of mercury on the digestive tract

At 0.1 μg $HgCl_2$/l/4months dose, the esophagus has the cells generally with normal aspect. Sometimes we observed the presence of completely destroyed cells. Also, at this dose, the intestinal epithelium appears degraded.

At 1 μg $HgCl_2$/l/4 months dose, the intestinal epithelium is destroyed completely in its length. The nuclei are more spread. Rarely, in some zones the epithelium is intact.

At 5 μg $HgCl_2$/l/4 days epithelium of the esophagus and stomach appear relatively complete. In the cytoplasm of goblet cells, the mercury accumulation is more pronounced in their apical pole.

Also, in the connective and muscular tissue there are presented the mercury granulations. The little mercury granulations are observed also in the amoebocytes of the connective tissue.

Sometimes, at the same dose, the intestinal epithelium appears interrupted. The nuclei of the ciliated cells have different forms and they are pycnotic in majority cases. In these cells we remark numerous mercury granulations. In the connective cells there exist a lot of mercury granulations and the form of nuclei is modified. The muscular cells have flat very chromatic nuclei. In these cells the mercury accumulation is less pronounced than in the connective tissue.

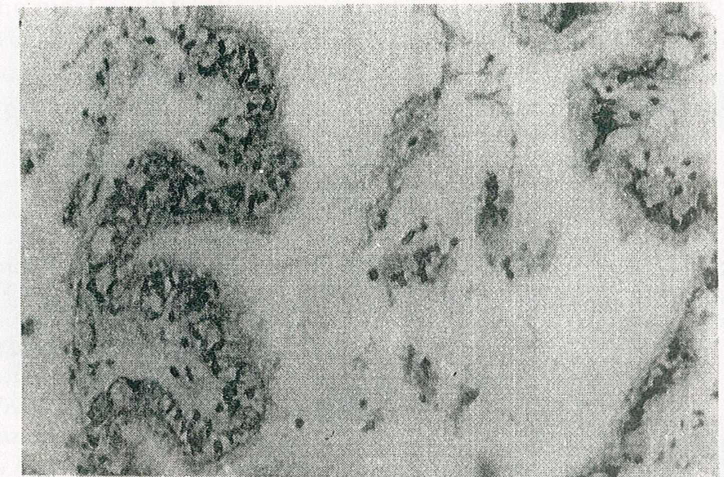

Fig. 1. – Normal aspect of intestine.

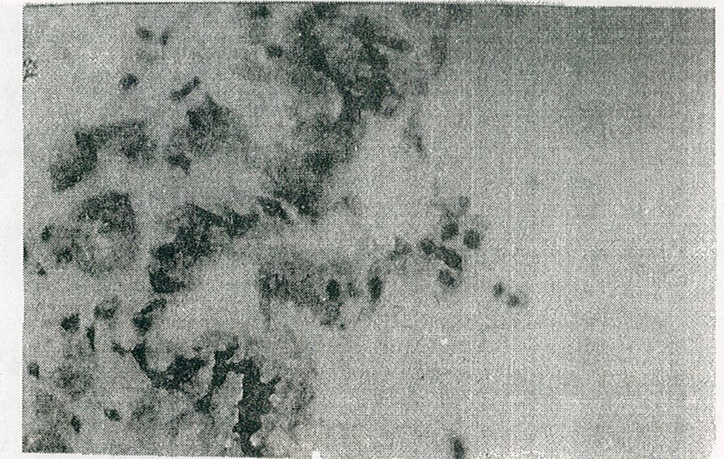

Fig. 2. – The epithelium of the stomach after treatment with 0.1 μg $HgCl_2$/l/4 months.

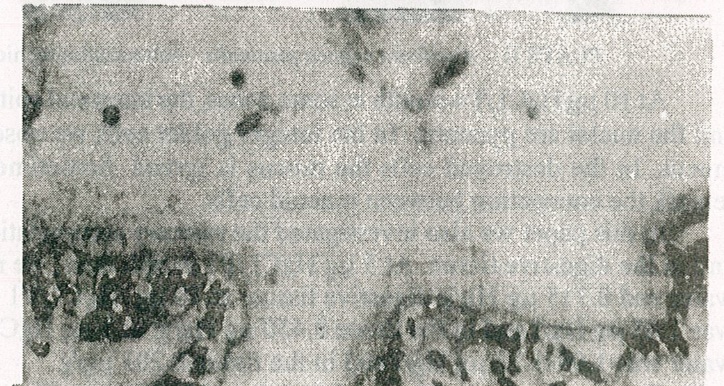

Fig. 3. – The epithelium of the intestine after treatment with 0.1μg HgCl2/l/4months.

PLATE I – *Anodonta cygnea piscinalis:*

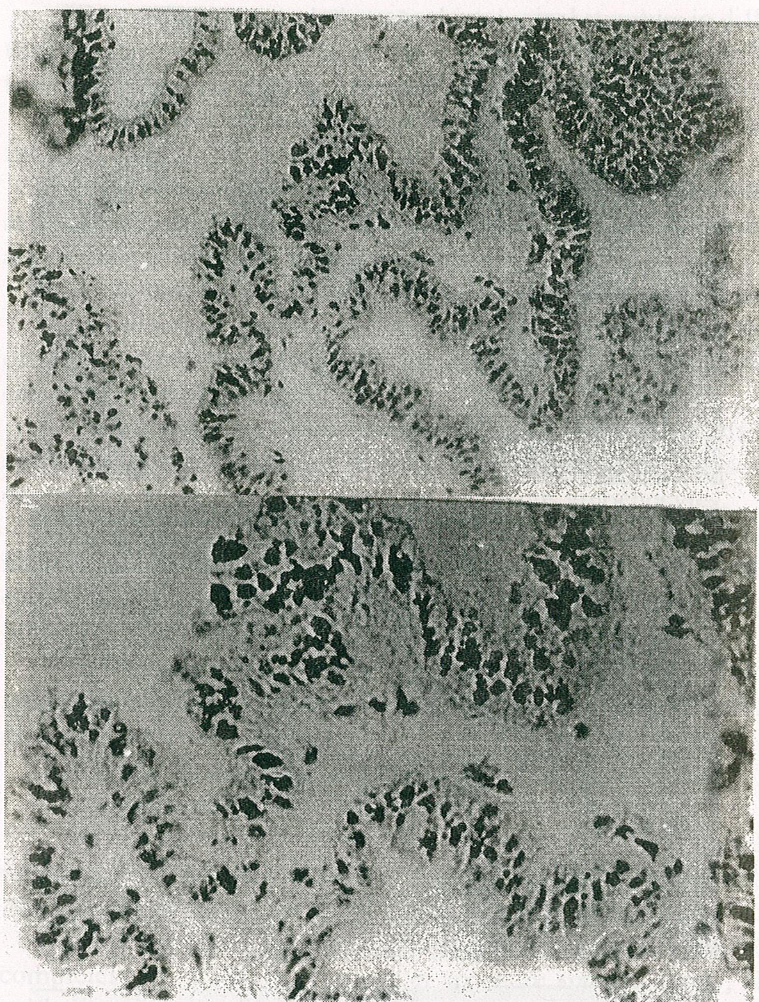

Fig. 4. – The mercury granulations in the cells of the stomach after treatment with 5 µg $HgCl_2$/l/4 days.

Fig. 5. – The mercury granulations in the cells of the intestine after treatment with 5 µg $HgCl_2$/l/4 days.

PLATE II – *Anodonta cygnea piscinalis* - autometallographic method:

At 10 µg $HgCl_2$/l/4 month in some zones, the intestinal epithelium is destroyed and the nuclei are pycnotic. In the integer goblet cells we observed the unaltered mucus. In the destroyed cells the mucus is spread. Sometimes, at this dose, we remark the connection between mucoid cells.

In this paper we also investigated the mercury accumulation both in the water and in the digestive tissue. At 5 µg $HgCl_2$/l/1 month dose, we notice 1.70 µg Hg/l water and 0.315 µg Hg/g digestive tissue; at 10 µg /$HgCl_2$/l/1 month in the water was 1.63 µg Hg/l and in the tissue 0.450 µg Hg/g; at 1 µg $HgCl_2$/l/4 months in the water was 1.09-1.12 µg Hg/l and in the tissue 0.205 µg/g.

Our results at *Anodonta piscinalis* releaved that all utilised intoxication doses modified the epithelium of the digestive tract. These modifications led to the total disintegration of the epithelium.

In general the most modifications produced by some heavy metals (Pb, Cd and Cu) were studied on the digestive and antennal glands (1,6).

The observed alterations on *Anodonta piscinalis* were similar to those on Crustacea (Crangon crangon, 1), on aquatic Gasteropoda (2) and other invertebrates.

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THE DYNAMICS OF ADRENAL-MEDULLARY CATECHOLAMINE CONTENT FOLLOWING UNIQUE AMINO-PHYLLINE (THEOPHYLLINE-ETHYLENE-DIAMINE) ADMINISTRATION IN MICE

ELENA POPOVICI, ANCA-PETRESCU RAIANU and R. POPA

Male adult mice were administered aminophylline (theophylline-ethylene diamine) in a single dose of 150 mg/kg body weight and sacrificed at different time intervals within 48 hours after drug injection. The medulla of adrenal glands was analysed histochemically for total catecholamines and noradrenaline content. The catecholamines clearly decreased after treatment. In contrast, no change in the noradrenaline content was noted. The loss of catecholamines began 30 min after the administration of aminophylline. It was most pronounced at 2 hours and maintained still evident until 48 hours after injection. The morphometric analysis showed that adrenal-medullary catecholamines decreased on average from 78% to 15% of gland volume, while noradrenaline varied not significantly (maximum variation of 11%) around the control value (12%). 48 hours after aminophylline injection some of group animals showed still low catecholamine content (around 20%). None of 51 investigated glands showed complete depletion and the levels of remained catecholamines always exceeded the correspondent percentage of noradrenaline. The results suggest that aminophylline had stimulated selectively the adrenaline release from the medulla of mouse adrenal gland.

INTRODUCTION

The aminophylline appears to affect significantly the function of adrenal-medullary cells by stimulating the catecholamine secretion as was previously reported (1), (15). However, only few investigations on this matter have been carried out (3), (16), (20).

The stimulatory action of aminophylline was clearly proved in perfused bovine glands by Poisner (15), who has utilised biochemical methods to evidence it.

In a previous study (17) performed "in vivo", we attempted to induce the same effect in mice. The catecholamines were detected in the light microscopy as dichromate-chromate and potassium iodate stained material. The stimulatory effect of aminophylline was estimated by the degree of catecholamine depletion. Only a slight decrease of catecholamines was observed while noradrenaline content was similar to the control.

Taking into account the apparently contradictory findings obtained "in vitro" (15) and "in vivo" (17) we considered useful to undertake the present quantitative investigation in which the stimulatory effect of aminophylline on adrenal-medullary catecholamine release has been examined within 48 hours.

REV. ROUM. BIOL.– BIOL. ANIM., TOME 40, N° 1, P. 39 – 49, BUCAREST, 1995

MATERIALS AND METHODS

Male adult albino mice of 23-28g raised in the Institute of Biology biobase were housed in groups of 6 animals in glass cages, for two weeks before the treatment and during the experiment. Feed and water were given "ad libitum". In 10 experimental groups (each of 5 or 10 mice) the "Miofilin" product (2.4% aminophylline aqueous solution) was administered i.p. in single 150 mg/kg. body weight dose. The mice were decapitated at the following time intervals: 10, 20, 30 min., 2, 10, 18, 24, 30 and 48 hours after injection. The mortality registered during the experiment is shown in Table 1. Both adrenal glands were removed from each animal and processed histochemically, one for total catecholamines and the other for noradrenaline, according to the dichromate-chromate and potassium iodate methods of Hillarp and Hokfelt (9), modified by Picard and Vitry (14) for paraffin embedded tissue.

6μ and 9μ serial sections obtained from entire medulla were examined in light microscopy for estimation of catecholamine quantity judged by the intensity of positive histochemically material. The glands which had revealed intense depletion (larger than 50%) were further analysed more precisely by using two test systems according to Weibel (24):

A - Square grid with 5 mm side squares having 408 test points;

B - Test system with 84 lines of 10 mm arranged in an equilateral triangular network.

5-6 sections, almost evenly distributed in one adrenal medulla, were chosen for quantitative testing. The 9/11cm micrographs were obtained with a 16× objective (microscope field comprised almost the entire medulla borders). The statistically significant differences between results were established by means of the F test and the Student test.

RESULTS

Adrenal weights

Relative weight of a single adrenal was about 10mg/100g.body weight and that of pairs about 20mg/100g.body weight at all mice groups (Table 1). The statistical analysis revealed no significant differences against control except for the right adrenal 30 hours after injection. It might be concluded that the aminophylline had no influence on the relative weight of mice adrenals.

Although immersed in different fixatives the left adrenals were greater than the right ones (Table 1). This also was reported by other authors (8), (23), (25), (26). The average weight of the right adrenal represented 89% of the left one. The statistical analysis indicated no significant difference.

Dichromate-chromate reaction

The positive material, which indicates the existence of both adrenaline and noradrenaline, was present throughout the medulla at control mice (Fig. 1) and 10,

Table 1

Adrenal relative weights at male adult mice treated with single 150 mg/kg body weight aminophylline dose

Group	Anim. nr.	Treatment	Time after inject.	Dead anim. nr.	Sacrif. anim. nr.	Body weight (g)	Adrenal relative weights (mg/100 g b. w.)*		
							Right	Left	Pairs
Controls	10	-	-	-	10	26.00±2.37	11.18±0.92	12.51±1.10	23.69±1.97
I	5	Aminophylline single dose (150 mg/kg b. w.)	10 min	2	3	25.66±1.45	10.79±1.48	11.95±1.21	22.74±2.52
II	5		20 min	1	4	28.75±1.49	9.05±1.03	12.83±0.91	21.88±1.01
III	5		30 min	1	4	23.50±0.86	10.49±1.43	11.76±0.70	22.26±1.92
IV	5		2 h	1	4	26.75±0.85	8.98±1.56	11.78±1.04	20.76±2.34
V	5		6 h	1	4	23.00±1.87	10.91±1.13	11.93±0.43	22.84±1.33
VI	10		10 h	2	8	26.25±1.20	12.33±1.38	10.83±0.94	23.16±1.90
VII	11		17 h	0	11	26.09±1.78	10.91±0.62	11.03±0.67	21.94±0.96
VIII	10		24 h	4	6	28.83±1.64	8.26±0.64	9.34±1.15	17.60±1.61
IX	10		30 h	2	8	26.00±1.19	8.04**±0.51	10.12±0.56	18.17±1.01
X	10		48 h	1	9	27.77±1.10	10.27±0.93	10.20±1.01	20.47±1.87

*Values represent averages for sacrificed animals (x + SE)

**Significant difference ($p < 0.01$) against control

20 and 30 min. after injection in the case of treated ones. The marked loss of stainable material from medullary cells within 2 hours after the drug injection supports that the depletion began later than 30 min. The catecholamine depletion remained quite pronounced until 48 hours after injection (Fig. 2).

A considerable variability of the individual response to the aminophylline administration was noticed. Thus V to X groups included both animals with intense depletion, as well as individuals with slight or even no loss of positive material.

Potassium iodate reaction

This method distinguishes clearly the noradrenaline from adrenaline cells by the presence of dark-brown, iodate positive material (Fig. 3). This reaction revealed in mouse medulla a small proportion of noradrenaline cells as compared with adrenaline ones, almost the same in control (Fig. 3) and all treated groups as in Fig. 4.

Morphometric analysis results

The above histochemical techniques have shown a high degree of specificity and consequently are suitable for test system quantitative methods.

The values obtained with A and B test systems are comparable, as can be seen in Table 2. The results presented further in this paper (Tables 3, 4) are means of the two test data.

Dichromate-chromate reaction. The maximum degree of depletion was noticed 2 hours after injection (Fig. 5, Table 3), whereas the complete loss of dichromate-chromate stainable material could not be observed at any studied time interval, even at the animals with intense depletion. The same fact was valid in every studied individual, as it is illustrated by examples from Table 4. The catecholamine content remained constantly quite low since 2 until 48 hours after injection, at mice with marked depletion (Fig. 5, Table 3).

Potassium iodate reaction. Quantitative analysis given a proportion of 12% noradrenaline cells in adrenal medulla at control mice and statistically not significant variations of this at treated groups (Fig. 5, Table 3).

In mice with pronounced depletion, catecholamine content was very close to that of adrenaline, at all time intervals (fig. 5). Also, in each studied mouse, the amount of catecholamine in medulla tissue after stimulation was never lower than the correspondent noradrenaline content (Table 4). In addition, the distribution pattern of the remaining dichromate-chromate positive cells was consistent with that of the iodate positive cells of the contralateral adrenal gland (Figs. 2, 4).

DISCUSSION

The present results on the relative weights of adrenal glands are comparable with those obtained by other authors, as was discussed in a previous paper (17).

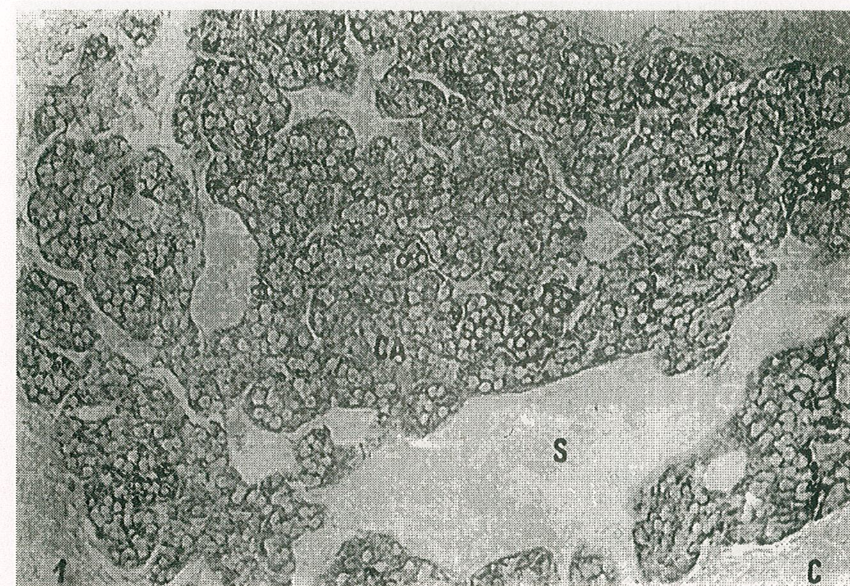

Fig. 1.-Adrenal medulla of male adult normal mouse. Dichromate-chromate reaction. Positive material (CA) indicating the presence both of adrenaline and noradrenaline, throughout the medulla tissue section. Cortical tissue (C) remains unstained. Many capillary sinusoids (S).

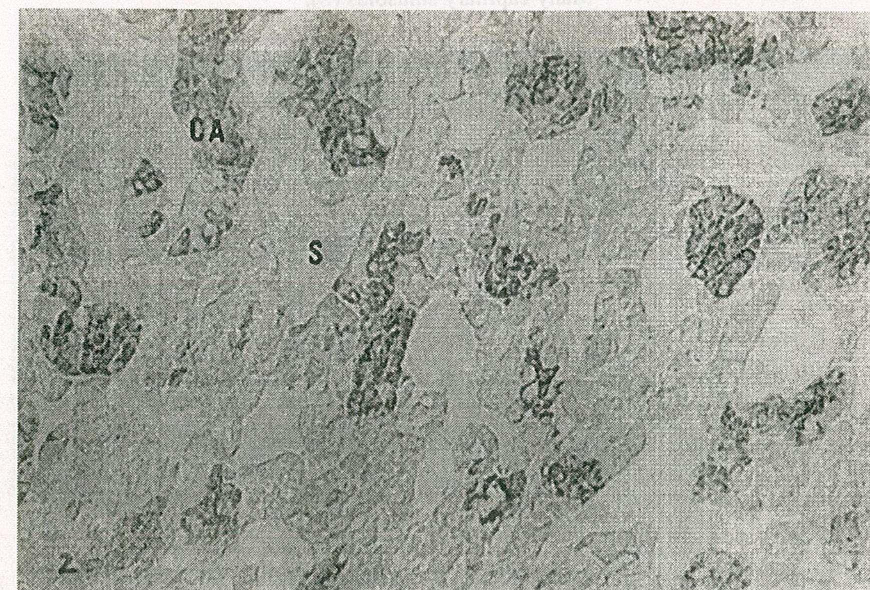

Fig. 2.- Adrenal medulla of male adult mouse 30 hours after a single dose of 150 mg/kg.body weight aminophylline. Dichromate-chromate reaction. Small positive material areas indicating the presence of catecholamines (CA) in cells. Cortical tissue (C) gave negative reaction. Many capillary sinusoids (S).

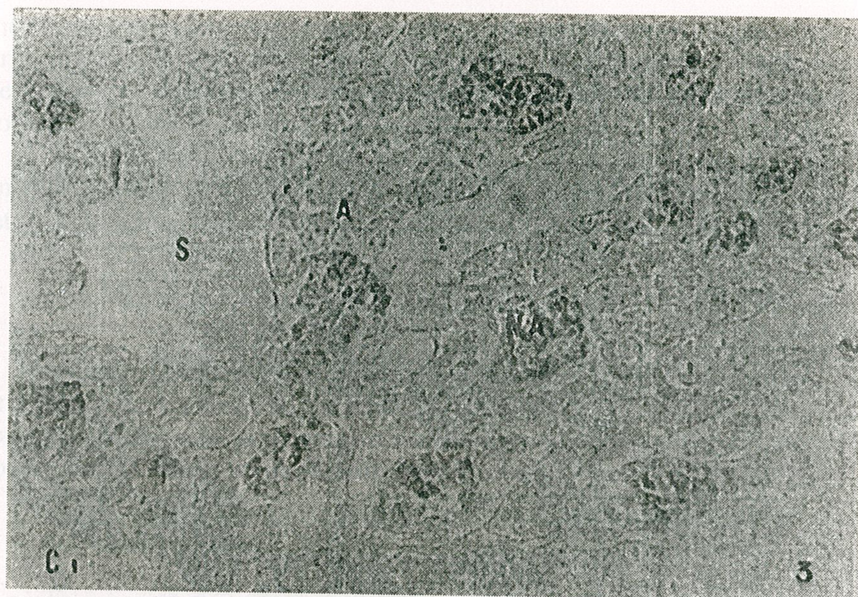

Fig. 3. Adrenal medulla of male adult normal mouse. Potassium-iodate reaction. Positive material islets of variable dimensions indicating the presence of noradrenaline (NA) in cells, randomly distributed in medulla tissue. Adrenaline (A) and cortical (C) cells remain unstained. Many capillary sinusoids (S).

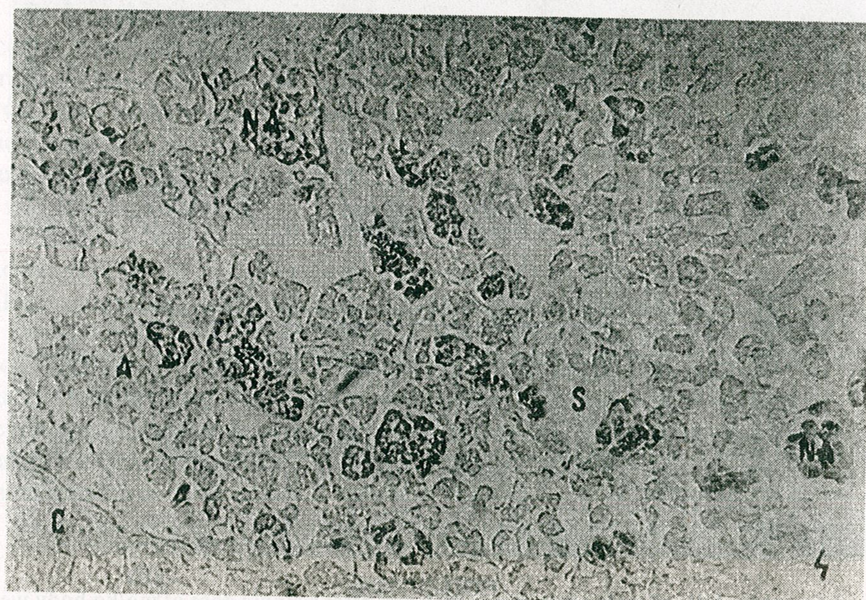

Fig. 4. Adrenal medulla of male adult mouse 30 hours after a single dose of 150 mg/kg.body weight aminophylline. Potassium-iodate reaction. Same pattern as normal mouse.

Table 2

The comparison between morphometrical methods using A and B test systems.

Conditions	Micrograph. (Tissue section) No.	Capillary sinusoids (%)*		Cathechol. tissue (%)*	
		A TEST	B TEST	A TEST	B TEST
Mouse sacrified at 2h	1	18.3	17.4	13.7	10.4
	2	21.3	20.7	12.5	12.9
	3	4.3	4.0	10.9	11.6
	4	16.1	18.1	7.1	8.1
	5	23.5	19.7	4.5	4.4
	Average	16.7	16.0	9.7	9.5
Mouse sacrified at 6h	1	29.2	30.9	15.9	17.9
	2	3.5	4.5	23.2	26.0
	3	17.0	7.5	13.3	21.4
	4	9.14	10.2	21.7	20.7
	5	4.4	5.1	18.2	22.0
	Average	10.6	11.6	18.5	21.6
Mouse sacrified at 10h	1	9.3	5.8	24.7	21.5
	2	17.1	15.5	19.7	19.3
	3	15.6	14.6	14.5	16.4
	4	36.1	36.1	21.6	20.1
	5	34.1	34.3	19.8	20.1
	Average	22.4	21.3	20.1	19.5
Mouse sacrified at 30h	1	8.4	7.9	19.3	22.3
	2	22.7	19.9	7.8	11.4
	3	21.4	22.1	6.2	5.7
	4	20.5	18.8	16.3	15.5
	5	27.3	27.1	15.0	17.2
	Average	20.1	19.2	12.9	14.4

*Values represent adrenal medulla volume percentages

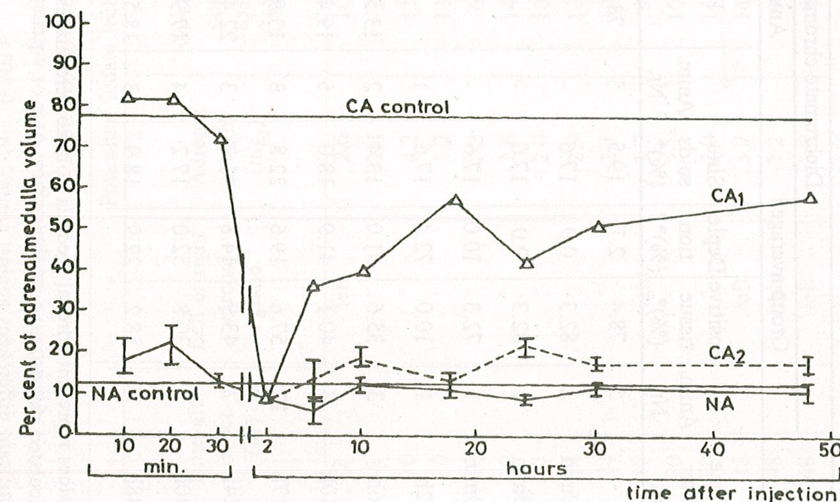

Fig.5. The dynamics of adrenal catecholamine content induced by a single dose of 150 mg/kg. body weight aminophylline in male adult mice.

CA_1 Δ--------Δ Total catecholamine content (group averages)
CA_2 ------------ Total catecholamine content (averages for animals with intense depletion)
NA -------------- Noradrenaline content (group averages)

Table 3

Quantitative morphological data of adrenal medulla male adult mice treated with single 150 mg/kg. body weight aminophylline dose

		Dichromate-chromate reaction								Potassium iodate reaction			
		Group averages				Animals with intense depletion				Group averages			
Group No.	Time after inj.	Anim. Nr.	Positive tissue (%)*	Depletion (%)**	Sinusoids (%)*	Anim. Nr.	Positive tissue (%)*	Depletion (%)**	Sinusoids (%)*	Anim. Nr.	Positive tissue (%)*	Nonpositive tissue (%)**	Sinusoids (%)*
Control	-	5	78.4	2.1	19.5	5	78.4 ± 3.98	2.1	19.5 ± 3.77	5	12.2 ± 1.73	76.4	11.4 ± 2.93
I	10min	2	82.3	0.0	17.6	-	-	-	-	3	18.7 ± 3.95	58.6	22.7 ± 6.41
II	20min	1	82.3	0.0	17.6	-	-	-	-	3	21.7 ± 4.53	60.9	17.4 ± 0.83
III	30min	1	72.3	10.0	17.6	-	-	-	-	2	13.9 ± 0.86	73.7	12.4 ± 4.57
IV	2h	1	10.0	72.4	17.6	1	9.9	73.3	16.8	3	8.9 ± 1.14	73.8	17.3 ± 7.44
V	6h	3	35.6	51.0	13.4	2	13.5***±5.85	75.2	11.3 ± 0.19	2	6.4 ± 3.28	72.1	21.5 ± 7.88
VI	10h	8	40.1	41.9	18.0	5	19.4***±1.56	62.5	18.1 ± 1.78	7	12.4 ± 1.48	77.0	10.6 ± 1.79
VII	17h	12	57.6	19.6	22.8	3	13.9***±0.91	60.5	25.6 ± 1.67	4	11.2 ± 2.00	68.0	20.8 ± 5.82
VIII	24h	7	43.9	14.8	41.2	3	22.4***±1.75	66.4	11.2 ± 1.28	3	9.2 ± 0.94	67.4	23.3 ± 4.85
IX	30h	8	30.8	52.0	17.2	4	17.9***±1.62	65.3	16.8 ± 2.28	6	11.6 ± 0.77	71.7	16.7 ± 2.58
X	48h	8	58.2	22.9	18.9	3	18.5***±2.17	60.4	21.1 ± 2.76	6	11.4 ± 1.16	73.1	15.5 ± 2.17

* Values represent adrenal medulla volume percentages
** Calculated values
*** Significant differences against control ($p < 0.001$)

Table 4

Quantitative morphological data of adrenal medulla pairs from the same mouse treated with single 150 mg/kg. body weight aminophylline dose

		Potassium iodate technique				Dichromate-chromate technique			
Anim. No.	Time after inject. (hr)	Left or right adrenal gland	Adrenal weight (mg.)	Noradren. cont. tissue (%)**	Capillary sinusoids (%)**	Left or right adrenal gland	Adrenal weight (mg.)	Catechol. cont. tissue (%)**	Capillary sinusoids (%)**
1	2	left	2.6	10.1	12.8	right	2.2	9.6	16.3
2	6	"	2.4	3.1	13.3	"	2.7	7.7	11.1
3	10	"	4.2	11.0	12.8	"	3.0	16.6	18.9
4	10	"	3.2	11.9	15.9	"	3.2	15.3	13.0
5	10	"	2.2	6.6	10.3	"	2.4	19.8	21.9
6	10	"	2.6	14.9	9.0	"	2.1	23.9	15.0
7	17	"	2.0	10.8	9.6	"	2.4	15.7	27.8
8	24	"	2.9	10.1	18.1	"	2.3	24.6	12.1
9	24	"	2.1	10.1	18.5	"	2.2	23.7	12.8
10	30	right	1.7	9.7	21.5	left	2.6	21.2	10.5
11	30	"	2.1	10.0	20.0	"	2.6	13.7	19.8
12	30	"	2.0	14.4	9.2	"	2.7	19.3	20.7
13	48	left	3.7	14.1	18.3	right	2.8	21.7	24.9
14	48	right	2.2	9.2	15.1	left	2.5	14.3	15.7
15	48	left	3.8	9.3	24.2	right	3.8	19.4	22.5

**Values represent adrenal medulla volume percentages

"In vivo" administration in mice of a single aminophylline dose (150mg/kg.body weight) caused massive catecholamine release from adrenal medulla gland, as evidenced by the dichromate-chromate histochemical method. This is in agreement with the biochemical results of other authors (15), (16). At the same time, the noradrenaline gland content was not affected by the treatment, as potassium iodate reaction had revealed. The above findings suggest that the loss of catecholamines was mostly due to the adrenaline release from the mouse medulla. This supposition is confirmed by morphometric analysis which had revealed that proportions of remained catecholamines after stimulation had never been smaller than the correspondent noradrenaline content, in every studied gland (Table 4). It seems that the aminophylline had stimulated selectively the adrenaline secretion of cells. Similarly, Douglas et al. (7) reported preferential release of adrenaline from the adrenal medulla induced by muscarine and pilocarpine.

In our experiment aminophylline did not cause complete depletion of catecholamines although a high, letal dose of drug was used. In this context, Vitry (22) observed a complete loss of dichromate-chromate stainable material, with reserpine and insuline. Wood et al. (27) using other histochemical methods, also obtained complete catecholamine depletion of adrenal medulla, with reserpine. It is possible that the aminophylline stimulate adrenal-medullary cells by a specific mechanism since Poisner (15) found that even in the absence of calcium ions, it still had evoked catecholamine release. It is interesting that serotonine, histamine and tirosine share the same property (10), while most stimulatory substances of adrenal medulla secretion, as bradykinine (5) are Ca^{++}-dependent.

Our present attempt of applying morphometrical methods to catecholamine specific histochemical techniques was undertaken in conformity with Weibel's (24) opinion that in this way it is possible to obtain reliable results. In the latest decades, the morphometric studies at light (19) and electronic microscopy level have proved their importance especially in pathology (2) and therefore their number gradually increases. Many refer to lung morphometry (4), (6) or ultrastructural features (11), (13). Some deals with adrenocortical tissue (11), (13), (18), (21), whereas very few are concerned with adrenal medulla. Nemes (12) applying histo-morphometric analysis found 8.98% noradrenaline cells in the internal medulla of rats and 7.9% in the external one. These values are comparable with 12% noradrenaline cells at control mice in the present report.

CONCLUSIONS

– Aminophylline (Theophylline-ethylene diamine) administered in male adult mice in a single 150mg/kg.body weight dose induced the loss of dichromate-chromate stainable material from the adrenal medulla, in a proportion of maximum 70% of the gland volume. This proves its stimulatory effect on catecholamine release from the adrenal medulla of mouse;

– Aminophylline did not influence the adrenal relative weight, noradrenaline cell percentage and capillary sinusoid volume;

–Aminophylline, even in a high letal dose, did not induce complete depletion of catecholamine in every studied adrenal medulla. The percentage of cells remained

dichromate-chromate reactive after treatment was never smaller than the corresponding noradrenaline cell percentage. It can be interpreted that the aminophylline had stimulated selectively the adrenaline release from the medulla;

– The applying of morphometric methods to dichromate-chromate and potassium iodate histochemical techniques may provide reliable quantitative data, in addition to biochemical determinations.

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THE ACTION OF SOME RESIN EXTRACTS ON THE WALKER CARCINOSARCOMA IN WISTAR RATS

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and IOANA ROMAN

The treatment of experimentally-induced Walker 256 carcinosarcoma in rats with some coniferous resin extracts, having different monoterpene content, produced modifications in the tumours which were dependent on the nature of the utilized fraction. The most obvious effects were noticed with fraction F-4 (the most rich in pinene), which reduced the volume and the relative weight of the tumours and also induced biochemical modifications at their level.

Coniferous resin extracts different compositions were shown to have immunostimulatory effects in mammals (2). As the immunologic process is affected in a tumour evolution, we investigated the effects of several coniferous resins on the development and on some biochemical modifications which take place consecutively to an experimental tumour inocculation in the Wistar rat. These extracts produced, in normal organisms, important modifications at the level of the organs implied in the immune response (7), (9), (10) and in thymocytes (8).

MATERIALS AND METHODS

Experiments were performed on male, adult, Wistar rats, weighing 160±5 g. Animals were raised in appropriate zoohygienical conditions, water and food being administered *ad libitum.* Rats were organised in four experimental groups, each group consisting of 8-10 animals, as follows: - a tumour.bearing control group (TC), and three other tumour-bearing groups, treated with coniferous resin extracts (F-4, F-5 and respectively F-7).

The Walker 256 carcinosarcoma was obtained from the Oncological Institute in Cluj-Napoca. The inducement of the carcinosarcoma was made by subcutaneous implantation, between the shoulder blades, using the following procedure: fragments of 3-4 mm^3 were taken from the cortical area of the tumour (not necrosed) and were implanted to the receiving animals, using a glass trocar. 10 days after the inoculation, when tumours were well-developed, the treatment with resin extracts was started. Extracts were administered by peritumoral injections, for 7 days, starting from the 11th day after tumour inoculation. For each resin fraction, the daily dose was 1/10 from its LD_{50}, as previously established (7).

Rats were sacrificed by decapitation, after a previous fasting period of 16 hours. Tumours were immediately taken, weighed on a torsion balance and their length (L) and width (W) were measured, in order to calculate the tumour volume, expressed in cm^3. Tumoural tissue samples were used to determine the total protein content (4), the free amino-acids nitrogen (5) and the concentrations (6) of nucleic acids (DNA and RNA).

REV. ROUM. BIOL. – BIOL. ANIM., TOME 40, N° 1, P. 51–53, BUCAREST, 1995

Mean values were calculated for each biometrical and biochemical parameters and the means tested for homogeneity by Chauvenet's criterion. Student's "t" test was used to check the statistical significance of the differences between means; a significant threshold level was considered at $p = 0.05$.

RESULTS AND DISCUSSIONS

Inoculated animals developed tumours in a proportion of 98%, some of them bearing even 2 or 3 tumours. As can be seen in Table 1, the direction of the modifications induced by the three resin extracts was the same, but it had different intensities. The relative weight of the tumours in the resin-treated groups was lower as compared to the control group, the difference being statistically significant in the F-4 and F-7 groups. The tumour volume significantly decreased in the F-4 group only, the greater values found in the other treated groups being not statistically confirmed. There was a significant raise of the total protein content in the tumours of the F-4 group, the group which showed an important decrease of the tumoural volume and relative weight. In this group the increase of the protein concentration was paralleled by higher values of the free amino acids nitrogen, as compared to the control group. Nitrogen concentration also raised in the F-5 and F-7 groups, although in these groups the protein level was not modified. It is remarkable that there wasn't any significant modification of the DNA and RNA content, in any treated group.

Table 1

The effects of three coniferous resin extracts on some biochemical and biometrical parameters of the Walker 256 carcinosarcoma in Wistar male rats

Groups:	C	F-4	F-5	F-7
TP (mg%)	192.09±11,48 -	313.80±28.66 *±63.36*	229.53±35.26 +19.49	195.20±18.04 +1.61
FAN (mg%)	96.78±15.83 -	161.61±24.36 *±66.98*	195.82±20.22 *+102.33*	175.54±19.19 *+81.38*
DNA (mg/g)	0.55±0.09 -	0.77±0.10 +40	0.68±0.20 +23.63	0.60±0.07 +9.09
RNA (mg/g)	0.61±0.08 -	0.76±0.20 +24.59	0.55±0.09 -9.84	0.73±0.20 +19.67
TV (cm³)	221.80±26.41 -	43.36±5.47 *-80.45*	357.60±100.87 +61.22	346.60±100.15 +56.30
RTV (g)	17.12±3.53 -	6.60±1.12 *-61.45*	11.60±2.40 -32.26	7.16±0.79 *-58.18*

Results are means±standard errors; percentage difference versus the control group (±D%); the statistical significant differences versus control group are underlined; TP=total protein; FAN=free aminoacid nitrogen; TV=tumour volume; RTV=relative tumoural weight. Other explanations in the text.

The obtained data emphasized different effects of the resin extracts in correlation with the chemical composition (alpha and beta pynen, camphen, caren, lymonen etc.) of each fraction.

We noticed that the F-4 fraction has stronger effects upon the tested parameters, as compared to the other fractions. The decrease of the tumour volume and of its relative weight seem to be in contradiction with the significant raise of total protein content and of free amino acid nitrogen. A possible explanation could be a modification of the water content in the tumours, which also changed the consistence of the tumoural tissue. It is difficult to give an appropriate interpretation of these phenomena, as the noticed effects are dependent on various factors (the administered resin dose, the duration of the treatment, the sex and age of the animals, etc.), which significantly influence the reactivity of the organism (3), (7), (8).

It is of interest that the modifications of the biochemical parameters, consecutive the resin treatment, were similar to those obtained on isolated thymocytes from rats treated with the same resin fractions and in the same dose (8), (9). This fact leads to the supposition that the thymus may be the principal lymphatic organ involved in the anti-tumoural mechanisms. Some endocrine glands, as thyroid (7), sex glands (3) and adrenals (unpublished data) also participate in the anti-tumoural response.

Our electronographic investigations (unpublished data) established that these resin extracts have electromagnetic properties. After Smirnov and Milcu (1), these properties could explain the particular biological process that takes place at the chemical and molecular level. Their effects target on the cell membrane and modifies the transmembrane potential. These observations emphasize some particular properties of the tested coniferous resin extracts with membranotrope character, that confer them various pharmacological and medical utilisations.

We may conclude that the anti-tumoural actions of the coniferous resin extracts were dependent on the nature of the fraction and affected both the biometrical and biochemical parameters of the tumours.

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HEPATIC GLUCOSE OVERPRODUCTION AND OTHER ENDOCRINE–METABOLIC DISORDERS IN FLUOCINOLONE–INDUCED HYPERGLYCEMIC WISTAR RATS

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In adult young male Wistar rats a 3-day lasting cutaneous treatment with Fluocinolone acetonid N-containing cream (50 mg 100 g b.w./day) induced hepatic glucose overproduction, reduction of Langerhans islet volumes, B-cell damages and exhaustion. These alterations were accompanied by adrenal atrophy, reduction of adrenocortical fasciculata and reticular zones and by thymolysis associated with lipid accumulation and characteristic cell alterations in the involuted remnant thymus. The hyperglycemia, hypercholesterolemia, elevated hepatic glycogenogenesis, increased serum alkaline phosphatase activity and reduced growth-rate of individuals are pleading for a glucocorticoid excess, realized by transcutan absorption of Fluocinolone acetonide, superpotent topical glucocorticoid.

Elsewhere we pointed out that cutaneously applied Fluocinolone acetonide N cream in white rats induces hyperglycemia, muscular insulinresistance, thymolysis, enhanced hepatic glycogenogenesis, adrenal atrophy, hypercholesterolemia, increased serum alkaline phosphatase activity and reduction in the growth rate of individual (5), (6), (8), (9), (10), (11).

In the present study the action of short-term cutaneous treatment with Fluocinolone acetonide N-containing commercial cream was examined upon the rate of glucose formation "in vitro" from glucose-6-phosphate in the presence of liver homogenate. At the same time, the glycemia, cholesterolemia, liver glycogen content, histological aspect of pancreatic Langerhans islets, adrenal cortex and thymus, as well as the activity of serum alkaline phosphatase and the growth-rate of animals were tested, these parameters being sensitive and characteristic indicators of glucocorticoid excess, realized by transcutan absorption of Fluocinolone acetonide N-cream in Wistar rats (5), (6), (8), (9), (10), (11).

MATERIALS AND METHODS

For experiments adult young male Wistar rats of 140-160 g were used from the stockfarm of our laboratory, which were kept under standard bioclimatic and feeding laboratory conditions.

REV. ROUM. BIOL.– BIOL. ANIM., TOME 40, N° 1, P. 55–60, BUCAREST, 1995

The treatment of animals with commercial Fluocinolone acetonide N-cream ("Antibiotice S. A.", Iaşi-Romania, containing 25 mg Fluocinolone Acetonide per 100 g excipient) was carried out for 3 consecutive days, by smearing 50 mg cream per 100 g b.w. per day in a thin layer at a surface of 2.0 cm^2 on the inguinal region of the skin. Both the normal and the treated animals were sacrificed by sectioning the carotid vessels and exsanguination, after a fasting period of 18 hrs, i.e. 24 hrs following the cessation of the treatment. Prior to sacrifice, the drinking water was provided *ad libitum*.

The rate of "in vitro" glucose formation from glucose-6-phosphate in the presence of liver homogenate was evaluated by using the G-6-Pase activity method of Harper (4), applying for glucose determination the GOD-Perid method of Werner *et al.* (15), and Test Combination Glucose Kit ("Boehringer Mannheim GmbH Diagnostica", Germany).

For obtaining liver homogenate, from each animal liver-pieces of 250mg were quickly excised and homogenized in 9.75 ml ice-cold 0.1M TRIS ("Austranal") solution (adjusted to pH=6.5 with 2N HC1), using a Potter-Elvejhem apparatus. The homogenate was filtered on Ny-110-HD-filter (Germany), 1.0 ml filtrate corresponding such to 25 mg liver extract. From the filtered extract 0.1 ml was incubated for 15 min at 37°C with 0.1 ml freshly dissolved 0.08M glucose-6-phosphate (alpha-D-Glucose-6-Phosphate-$Na_2 \cdot H_2O$ "Serva", in TRIS-HC1 buffer, pH=6.5). Simultaneously, control samples (0.1 ml filtered homogenate and 0.1 ml TRIS-HC1 solution), as well as blank samples (0.1 ml TRIS-HC1 solution and 0.1 ml G-6-P solution) were used for incubation. After incubation, the trials were deproteinized with 2.0 ml 5% trichloracetic acid and centrifuged for 5 min at 2,500 r.p.m. From 0.25 ml sample, control and blank sample the glucose content was determined with GOD-Perid Glucose method (15). On the basis of differences between the glucose content of the samples and control samples (red photocolorimetrically at 610 nm, against the corresponding blank samples, using a "Specol" apparatus). The rate of hepatic glucose formation from G-6-P was calculated and expressed in „μMoles per g liver per minute, at 37°C, using glucose (p.a. "Merck") standard solution and the formula of Harper (3), (4).

The fasting glycemia level was estimated with GOD-Perid Glucose Kit ("Boehringer" Mannheim GmbH, Germany), according to Werner *et al.* (15), the total serum cholesterol was assayed with the micromethod of Zlatkis *et al.* (16), the liver glycogen content with the method of Montgomery (12) and the activity of serum alkaline-phosphatase was measured with a Test-Combination Alkaline-P-ase Kit ("Boehringer"Mannheim GmbH, Germany).

The structure of Langerhans-islets, adrenals and thymus was examined microscopically on the basis of usual histological methods (1), (2), (13), while the degree of thymus involution, adrenal atrophy and of the spleen-weight reduction, as well as the growth-rate of individuals were evaluated by our procedures (5), (6), (7), (8), (9), (10), (11).

The results were statistically checked for the homogeneity of the means using Chauvenet's criterion. Mean values were compared according to Student's *t* test, P = 0.05 being accepted as the limit of significance of modifications against the control values.

RESULTS AND DISCUSSIONS

From the data (Table 1) one can see that in Fluocinolone-induced hyperglycemic rats the hepatic G-6-Pase activity is significantly enhanced, the rate of glucose formation"in vitro" from G-6-P in the presence of liver homogenate at 37°C and 6.5 pH-optimum was intensified with 133.34% as compared to the normal value ($P < 0.001$), the fasting glycemia level being arised with 91.04% vs. the control ($P < 0.001$). These data suggest the conclusion that in Fluocinolone-evoked hyperglycemia in Wistar rats, beside the muscular insulinresistance (8), (11), the hepatic glucose overproduction essentially participates. On the other hand, the results indicate that the use of GOD-Perid Kit for testing hepatic glucose formation "in vitro" from G-6-P in the presence of liver homogenate, is an adequate and rapid procedure.

Simultaneously with the hyperglycemia and elevated hepatic glucose formation, observed in Fluocinolone-induced rats, a significant increase of liver glycogenogenesis (1258%, $P<0.001$), hypercholesterolemia (49,48%, $P<0.001$) and elevated serum alkalinePase activity (21.74%, $P<0.02$), thymolysis (44.61%, $P<0.001$), adrenal atrophy (22.50%, $P<0.01$) as well as reduction of spleen-weight (56.97%, $P<0.001$) were observed (Table 2), associated with a reduced growth-rate of individuals (Table 3). These endocrine-metabolic disorders are pleading for a pronounced glycocorticoid excess, realized by transcutaneously absorbed fluocinolone acetonide (5), (6), (8), (9), (10), (11).

From the analysis of histological data it is obvious that in Fluocinolone-induced hyperglycemic rats the langerhans-islet volumes are markedly reduced and accompanied by hyperplasia, degranulation and morpho-functional exhaustion of many beta-cells (Fig.1). These alterations strongly suggest the possibility that in the hyperglycemic action of Fluocinolone acetonide N-containing cream, the insular B-cell damage is mainly involved.

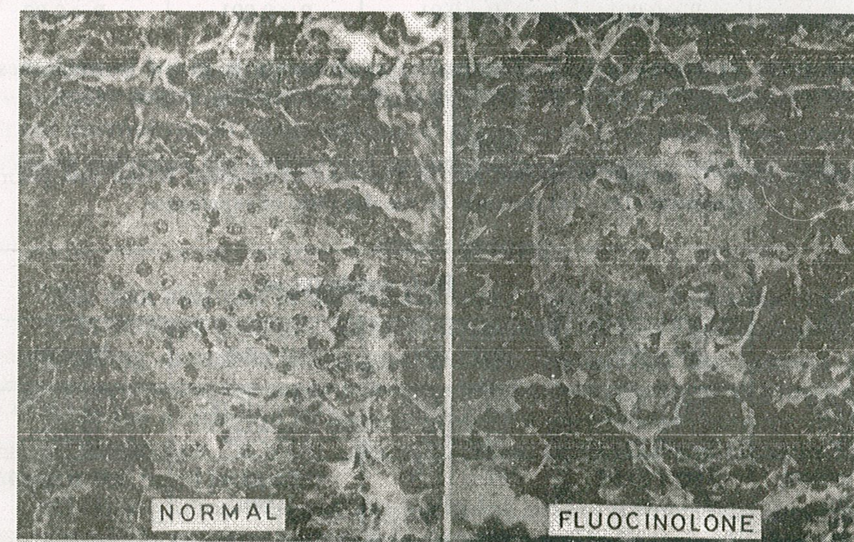

Fig. 1. Histological aspect of Langerhans islet in a normal and Fluocinolone–induced hyperglycemic Wistar rat.

Table 1

Hepatic glucose formation "in vitro", liver glycogen content, glycemia and serum cholesterol level in normal and Fluocinolone-treated rats

Groups	Hepatic glucose formation (µMoles/g/minute)	Liver glycogen (mg%)	Glycemia (mg%)	Serum cholesterol (mg%)
Normal	9.056 ± 0.668 (8)	421 ± 39.7 (7)	65 ± 1.35 (8)	220 ± 2.86 (8)
Fluocinolone	21.133 ± 0.851 (9) +133.34% $P < 0.001$	5114 ± 116.2 (8) +1257.39% $P < 0.001$	124 ± 3.73 (9) +91.04% $P < 0.001$	328 ± 10.12 (9) +49.48% $P < 0.001$

(The values represent means ± S. E. The number of experiments is given in brackets. Percent modifications and *P* are calculated vs. normal values).

Table 2

Serum alkaline phosphatase activity and relative weights of the thymus, adrenals and spleen in normal and Fluocinolone-treated Wistar rats

Groups	mg per 100g body – weight of			Serum alkaline Pase activity (mMoles/ml serum)
	Thymus	Adrenals	Spleen	
Normal	237 ± 10.6 (8)	22.09 ± 1.0 (8)	478 ± 20.7 (8)	99 ± 6.55 (8)
Fluocinolone	131 ± 6.5 (9) -44.61% P < 0.001	17.12 ±1.2 (9) -22.50% P < 0.01	273 ± 15.6 (9) -56.97% P 0.001	120 ± 5.08 (9) +22.74% P < 0.02

(Data are given as means ± S. E. Number of experiments is given in parentheses. Percent modifications and P are calculated vs. the corresponding normal values).

Table 3

The dynamics of body weight in normal and in Fluocinolone-treated Wistar rats prior to experiment (1st - 3rd day) and on the day (4th) of experiment

Groups	Body weight (g) on			
	1st – day	2nd – day	3rd – day	4th – day
Normal (8)	143 ± 9.34 -----	158 ± 10.42 +10.46%	160 ± 10.78 +11.70%	153 ± 9.96 +6.64%
Fluocinolone (9)	160 ± 13.04 -----	162 ± 13.36 +1.32%	158 ± 13.67 -1.39%	145 ± 12.64 -9.58%

(The values represent ± S. E. Number of experiments is given in brackets. Percent modifications are calculated *vs.* the corresponding initial values (1st - day).

Moreover, the significant thymolysis associated with a marked lipid content (of about 4-fold vs. normal; Fig. 2) and with characteristic cell-alterations in the involuted remnant thymus, as well as the adrenal atrophy accompanied by the reduction of adrenocortical fasciculata and reticular zones in Fluocinolone-treated rats clearly demonstrate that this superpotent synthetic fluorated topical glucocorticoid (dermocorticoid) causes a strong inhibition at the level of hypophyseal-adrenocortical axis,

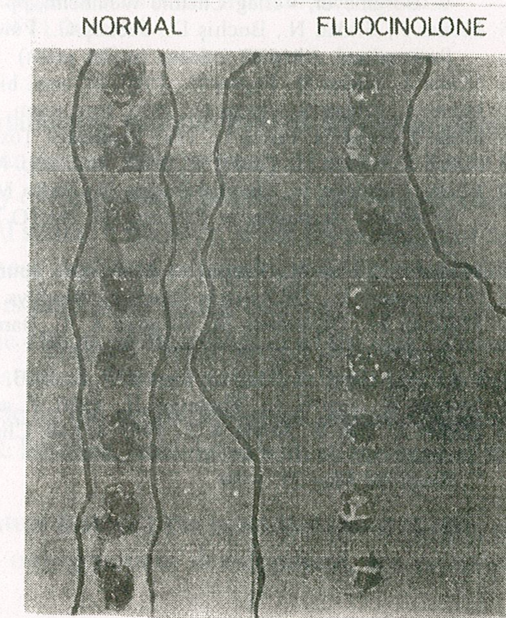

Fig.2 – Lipid release from normal and Fluocinolone–treated Wistar rat thymuses, placed and dried on chalk–paper at room temperature (contoured spots).

as reflected by thining of fasciculata and reticular zones in the adrenal cortex. In this context, our findings are in a good agreement with the recent dermological observations of Walsh *et al.* (14), that superpotent topical steroids suppress the activity of hypothalamus-pituitary-adrenal axis.

CONCLUSIONS

The data obtained in the present study suggest the following conclusions:

1. In the hyperglycemic action of cutaneously applied Fluocinolone acetonide N-containing cream in adult young male Wistar rats the hepatic glucose overproduction and the pancreatic beta-cell damage are essentially involved.

2. In the evaluation of hepatic glucose-6-phosphatase activity and hepatic glucose production "in vitro", the application of GOD-Perid Glucose Kit is an adequate and rapid procedure.

3. The glucocorticoid excess evoked by transcutan absorption of commercial Fluocinolone acetonide N-cream in adult male young Wistar rats exerts a strong inhibitory effect upon the hypophyseal-adrenocortical axis, leading at the same time to several endocrine-metabolic disorders.

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THE MANGANESE EFFECTS UPON SOME HEPATIC BIOCHEMICAL PARAMETERS IN CHICKENS

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Cornish-Rock chickens were treated with manganese at the age of 5 and 21 days after hatching. The modifications in the liver are dependent on the duration of treatment and on the age of chickens. The effect of manganese is more fast in the chickens treated at 5 days of age, and tardive in chickens of 21 days old.

Since 1975, Caron (3) have established that the liver is an organ among others with a large posibility to fix manganese. This property of the liver tissue is given by the intense metabolic activity of this tissue. The role of this oligoelement is proved especially in the pathological states of the liver, when the presence of the hepatoma is associated with a manganese depletion in hepatocytes. This depletion depends on the hepatoma nature and is associated with a decrease of the enzyme content in mitochondria (2, 5, 12). The effects of the excess or of the lack of manganese on the function of liver were studied. Our previous data have shown that the acute administration of manganese in the neuro-endocrine mature chickens, determines a stimulation of the hepatic function, associated with an increase of the total protein level (7).

Because there is no data in literature that we have consulted about studies on the normal liver in chickens in the ontogenetical development, we resumed our investigations on this topic.

MATERIAL AND METHODS

Experiments were performed on Cornish-Rock chickens aged 5 respectively 21 days at the beginning of the experiment. Chickens were maintained in our laboratory, in good zoohygienic conditions, water and age-fitted concentrated fodder were given *ad libitum*. The chickens were distributed in two groups, for both ages: control group (C) and treated group (M_n), which received manganese chloride (p.a.) as treatment. Each group contained 10 individuals. Manganese was given in a dose of 0.5 mg/kg fodder for 5 days chickens, respectively 1 mg/kg fodder for 21 days old chickens. Periods of treatment were 5 respectively 10 days. The fodder mixed with manganese was given daily.

Chickens were sacrified by decapitation, after a previous fasting period of 16 hours. Liver was immediately removed and used for the determination of total protein content (8), glycogen (11), free amino-acids nitrogen (15), nucleic

acids - RNA and DNA (16), fatty content (6) and the activities of alanine-aminotransferase (AlAT) and aspartate-aminotransferase (AspAT), (14).

Statistical processing of the results included the control of homogeneity of mean values by Chauvenet's criterion, aberrant values being eliminated and comparison of the means using Student's "t" test. Percentage modifications versus control values (±D%) were considered statistically significant for p = 0.05.

RESULTS AND DISCUSSION

As a first observation from the results of the experiment is that the hepatic modifications and the means of modification following the manganese administration depend not only on the period of administration, but also on the age, with some exceptions. Thus, hepatic modifications in 5 days old chickens are more obvious in 5 days of treatment chickens while in 21 days old chickens they appear after 10 days of treatment (Tables 1 and 2). Effects of manganese supplementation are explained by its action on the manganese superoxide dismutase enzyme. According to Borrello and co-workers (1) that effect might be due to an increase of the oxygenated radicals which can act upon the molecular "sensors".

Table 1

Liver reactions under manganese treatment in 5 days old chickens

Sacrif:	I		II	
Groups:	C	Mn	C	Mn
TP	411.83±54.10	+0.64	237.21±46.70	+50.73
G	3.81±1.14	-53.02	13.87±3.37	*+132.44*
Al.AT	1356.97±396.34	*+126.09*	2595.00±405.26	+11.32
As.AT	445.00±121.02	-7.01	1265.33±273.49	+2.22
NA	270.90±51.57	+16.89	335.16±37.72	-1.19
RNA	0.17±0.01	*+294.11*	0.13±0.01	*+123.07*
DNA	1.14±0.17	*+98.24*	0.32±0.08	+50.00
L	10.71±0.91	35.66	29.08±4.50	+8.21

Sacrifications: I - at 5 days of treatment; II - at 10 days of treatment; TP = total protein; G = glycogen; NA = free aminoacids nitrogen; L = lipids. In C group mean values ± standard error; in Mn group percentage differences versus C group. The statistical significant differences versus the control group are underlined. The values of TP are expressed in mg%; G in µg/mg; RNA, DNA, L in mg/g; NA in mg/100 g; AlAt and AsAT in µg/mg/h. Other explanations in the text.

Manganese can facilitate the generation of the reactive oxygen structures through the acceleration of the H_2O_2 formation (9), that can initiate the transcription of the manganese superoxide-dismutase enzyme. Formation of H_2O_2 can determine the transcription into DNA (2). This fact might explain our results obtained in 5 days old chickens, which at 5 days of treatment record an increase of RNA and DNA contents. The data of Borrello and co-workers (2) obtained on the tumoral cells show the important role of manganese upon the DNA level in the control of the superoxide-dismutase enzyme activity. Modifications of the transminase activity, especially in 21 days old chickens, at both periods of treatment, 5 respectively 10 days, are due to the action of this ion on the hepatocytes membrane permeability. Otherwise, since 1973 Chiarandini and Stefani (4) have pointed out that manganese can increase the polarization of excitable membranes. Although, there is affirmation that manganese intervenes in the fatty metabolism of the foetus (10, 13). Authors show that manganese is implied in the metabolism of lipoproteins with high density and the deficit of this oligoelement determines a decrease of these lipoproteins in rats. We find that the effects of the supplementation of fodder with this ion at chickens does not influence the content of hepatic fat (except for 21 days old chickens treated for 10 days, in which the content is significantly increase.

Table 2

Liver reactions under manganese treatment in 21 days old chickens

Sacrif:	I		II	
Groups:	C	Mn	C	Mn
TP	334.32±32.60	-8.31	355.27±37.76	+15.28
G	29.49±15.31	+39.97	84.41±11.46	*-81.31*
Al.AT	1393.22±167.54	*+48.40*	1475.89±48.53	+19.87
As.AT	680.48±184.30	-15.62	178.00±37.63	*+68.03*
NA	423.68±65.73	+36.16	133.48±2.45	*-28.30*
RNA	1.16±0.24	*-44.83*	1.02±0.16	-33.85
DNA	1.10±0.22	+31.81	0.99±0.25	*+19.47*
L	25.70±2.44	-3.21	7.17±0.69	*+60.41*

Explanations in Table 1 and in the text

In conclusion, the effect of manganese supplementation in the chickens fodder upon the normal liver is dependent on age and the period of administration.

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BIOCHEMICAL MODIFICATIONS IN THE THYMUS, THYROID AND ADRENALS FOLLOWING A THYROXINE AND LEUCOTROFINA TREATMENT IN THE FEMALE WISTAR RATS

IOANA ROMAN, RODICA GIURGEA, Z. URAY and MARTA GABOS

Treatment with thyroxine, in the female rats, determines an inhibition of the thyroid function followed by an adequate biochemical modification in the thymus and the adrenal. Leucotrofina treatment determines a typical stress state reflected by the adrenals reactions and a slight stimulation of the thyroid.

There exist a series of factors among which the thyroid hormones or the thymic extracts can influence the thymus and thyroid functions. The effects of the thymic extracts were studied both on mammals (1,3,24) and birds (9). The manner of action of the thyroid gland on the thymus was studied under different aspects on birds (8,9,14,22) and mammals (2,5,6) too. Previous data show the fact that the effects of these substances (thyroid hormones, thyroid inhibitors or thymic extracts) depend on both the dose and the way of administration and on the age of animal.

This paper is a sequel of our studies on this problem (10,11,19,20,21) and investigates some aspects regarding the thymus-thyroid relationship in rats.

MATERIAL AND METHODS

Experiments were performed on female Wistar rats, weighing 150±20 g. Rats were distributed in three experimental groups, of 8 animals each: - the *control group* (C), the *thyroxine treated group* (Tx) that received L-thyroxine (Thyrax-Organon, Belge S.A.), in a dose of 15 μg/100 g.b.wt., during 3 days, on the fourth day, the rats being sacrificed and the *leucotrofina treated group* (Le), (Ellem Milano, Italy), that received 3 days, in a daily dose of 0.1 ml (5 U.I./100 g b.wt.). On the fourth day animals were sacrificed. The substances were injected i.m. All groups have received ^{131}I, 24 hours before sacrification, i.p., in a dose of 1.0 μCi/animal. The rats were bred under adequate zoohygienic conditions, the food and water were given *ad libitum*. After 16 hours of inanition and etherization the animals were killed by decapitation. The thymus was immediately removed and after its weighing it was used for the biochemical modifications: total protein (TP), (13), free amino-acids nitrogen (AN), (18), nucleic acids - DNA and RNA - (23) and the glycogen (G), (17) contents. The weight of organ was noticed too. For adrenals it was determined the ascorbic acid content (Aa.), (15), the glycogen (G), (17) and the weight of organ. Thyroids of the rats that were injected with ^{131}I were collected and introduced in formol 10% for R.I.C. determination, and the blood serum was collected for T_3 and T_4 determination by R.I.A.

The data obtained were statistically interpreted, using Student's "t" test, and the aberrant values were eliminated according to Chauvenet's criterion. The percentual difference from the control group was also calculated (D%). The statistical significance was considered with $p = 0.05$.

RESULTS AND DISCUSSION

From the Table 1 result that the Tx has produced at the thymus level only significantly increase of the free aminoacids content, the other parameters having insignificant modifications. It is known that the thyroid hormones have effects on the protein metabolism, the effects being dependent on the dose administration. Thus, the high doses inhibit the protein synthesis and increase the free amino-acids content in plasma and tissues (20). Tx. do not produce a typical stress state, such it is result from the adrenal reaction (see Table 1). As concerns the incorporation degree of the ^{131}I in the thyroid, it can be remarked a significant decrease of the iodine incorporation to the Tx group. We presume that this inhibition given by the Tx treatment might be due to the raised level of the thyroid hormones (77.3% for T_3 and 551.09% for T_4), that determine an inhibition of the ^{131}I incorporation through a local feedback, as a result of the exogen contribution of the thyroid hormones (4). The most raised concentration of T_4 can be due to the fact that t 1/2 of that hormone is longer (8 days) than T_3 (1 day). The T_3 is the active hormone, a fact well known in the literature.

Table 1

Effects of thyroxine (Tx) and leucotrofina (Le) treatment on the thymus, adrenals, thyroid and thyroid hormones

Groups:	C	Tx	Le
		THYMUS	
TP (mg%)	345.27±63.17	-5.32	+22.69
AN (mg/pr.)	3.03±0.53	*+69.30*	+59.07
RNA (mg/g)	2.73±0.67	-19.22	+13.66
DNA (mg/g)	2.82±0.57	-33.90	+2.60
G (μg/mg)	1.16±0.22	-29.18	*-57.68*
OW (mg)	175.55±20.35	+4.24	+19.90
		ADRENALS	
G (μg/mg)	0.94±0.12	-3.19	+14.89
Aa. (μg/mg)	1.94±0.23	-17.01	*-45.36*
OW (mg)	26.85±0.98	*+38.32*	*+28.49*
		THYROID	
R.I.C. (%)	27.90±1.77	*-48.79*	+19.36
		BLOOD SERUM	
T_3 (ng/ml)	1.39	+77.30	+52.39
T_4 (ng/ml)	2.83	+510.09	+64.14

OW = organ weight; for control group = mean value ± standard error; for treated groups = percentage differences versus C group; statistical significance, at p = 0.05, is underlined. Other explanations in the text.

The modifications caused by leucotrofina treatment at the thymus level consist in the significant increase of the free amino-acids nitrogen content and an insignificant increase of the TP, DNA and RNA content. It comes out that it is a slight stimulation of the proteic metabolism resulted following a direct action of the thymic extract upon the thymus, a phenomenon found by the other authors too (7). Le. determine a significant decrease of the glycogen content in the thymus, probably because of its utilisation like power material in different synthesis. The thymic extract produced a typical state of stress on the body praised by a significant decrease of the ascorbic acid content and a significant increase of the organ weight, but insignificant for the glycogen content. This stress state produced by Le. was seen also in other previous experiments done in our laboratory (11,19). These modifications show a rise synthesis of the glucocorticoid hormones that have negative effects upon the thymus, in which exist receptors for glucocorticoids (12). Administration of Le. determines an insignificant increase of the R.I.C. and an increase of the thyroid hormones, fact evidenced by the other authors, which have shown that the thymus extracts determine an activation of the thyroid with an R.I.C. increase. That activation is expressed through a structural modification at the thyroid level (16).

In conclusion, Tx. determines an inhibition of the thyroid function being determined by the excess of the circulating thyroid hormones, expressed through a decrease of the protein content and an increase of the free amino-acids level. Le. determine a typical stress state, reflected in the adrenals reaction and less on the thymus, with a slight stimulation of the thyroid.

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ENERGETICAL BUDGET OF THE BACTERIOPLANKTON IN DÂMBOVIŢA LAKE

DOINA IONICĂ

Sont présentés les paramètrés du budget énergétique du bactérioplancton dans la période mars-août 1989 dans le lac Dâmboviţa. On a déterminé expérimentalement dans le laboratoire et «in situ» la respiration du bactérioplancton par la consommation bactérienne d'oxygène dans la masse d'eau et le temps de génération «g» des bactéries hétérotrophes.Les résultats montrent que 82% du budget énergétique représente la respiration du bactérioplancton et 18% - la production.De même, les coefficients qui caracterisent le flux énergétique: P/B=4,75, B/P=5, R/B=21, A/B=26, K_2=13 ont demontré que le bactérioplancton du lac Dâmboviţa représente un anneau trophique important dans la masse d'eau qui métabolise une quantité marquée de la matière organique avec une grande intensité et efficience jusqu'aux éléments minéraux.

The bacterioplankton, as a participant to energetical budget of a lacustrine ecosystem, has an important contribution not so much by its biomass or production but by its own activity to the biocenosis. As a decomposer of organic matter, the bacterioplankton represents the motive force of biogeochemical cycles, which means transfer of energy and matter.

MATERIAL AND METHOD

To estimate the energetical budget of the bacterioplankton there were used specific methods both in the field and in the laboratory.

To establish the parameters of the energetical budget of the bacterioplankton, the total energy of the ingested food (C) is assumed to have the same value with the assimilated energy (A) (1), because bacterial digestion is external. Thus, all micromolecular products of enzymatic decomposition which enter the cells represent assimilated food. There are no egesta and excreta like in the case of other organisms.

In conclusion: C=A

A part of assimilated energy is used in different forms of cellular activities such as: maintenance of cell integrity, chemical synthesis, active transport, conversion of food, degradation in heat, energy cumulated in the respiration process (R) and the other part is included in the cell mass, representing net production of this trophic level (P), and transmitted to consumers (5).

$A = P + R$

The transformation coefficients used are:

1 mg O_2 = 0.375 mg C (Ivlev, 1939)

Respiration and generation times of bacteria were measured experimentally.

There were determined both the respiration (which means also destruction of organic matter (D)) for every planktonic component and the chemical oxidation using selective inhibitors such as oxytetracycline and $HgCl_2$.(3).

In this case: $P = D\ (mgO_2/l/day) \times 0.08$ (4)

The generation time (g) is the period of time necessary to double the cell number in a population. The calculation formula of this parameter is:

$$g = \frac{t \log 2}{\log B_0 - 10gB_f} \qquad (1,4)$$

According to Gak[1], to "g" value experimentally calculated corresponds a constant K of the P/B ratio (turnover rate). Thus:

$P/B = K \rightarrow B = P/K$

To characterize the energy flow through the bacterioplankton there were determined:

- P/B
- B/P = the time for biomass replacement
- R/B = metabolization rate
- A/B = the speed of the energetical flow through the biomass unit in a unit of time

$K_2 = P/A \times 100$

K_2 = The efficiency of the microbial biosynthesis (the percent ratio of the amount of organic matter assimilated by microorganisms to the total amount of organic matter involved in the microbial metabolism) (4).

RESULTS AND DISCUSSIONS

The experiments took place during March-August 1989.

The results are presented in Table 1 (average values).

The analysis of the energetical parameter of bacterioplankton emphasizes interesting aspects of the energetical efficiency. Approximately 82% from the assimilated energy is converted to heat and lost in life processes (respiration) and only 18% is used for production. A high quantity of energy is used in the respiration process. Thus, a great amount of organic matter is decomposed and mineralized with high efficiency in aerobiosis. R/B ratio also demonstrates this.

K_2 value (13%) shows that the bacterioplankton of Dâmbovița lake plays an important role in the mineralization of organic matter, in the recycling of mineral elements.

The turnover rate and the high value of the flow speed demonstrate that about half of biomass (47%) is replaced in 5 hours.

The bacterioplankton from Dâmbovița lake, an important trophic level of water body, uses a great amount of organic matter from other trophic levels and metabolizes it intensively and efficiently to mineral elements.

In conclusion, the energetic flow assessment through the bacterioplankton and the characteristics of the energetical budget of the phytoplankton represent in the integrated research a possibility of estimating the efficiency of food consumption by zooplankton (2).

Table 1

The energetical budget of the bacterioplankton in lake Dâmbovița in 1989

Month	Biomass	Production	Respiration	Assimilation	K_2 %	P/B	B/P (h)	A/B	R/B
III	0.0326	0.1792	0.847	1.0262	17	5.54	4.3	31.5	26.0
IV	0.1344	0.7574	3.007	3.7646	20	5.54	4.1	28.0	22.3
V	0.0729	0.4032	1.9292	2.3324	17	5.54	2.4	32.0	26.4
VIII	0.8059	1.9166	8.9866	10.9032	17	2.38	10.0	13.5	11.5
X_a	0.2614	0.811	3.70	4.51	13	4.75	5.2	26.2	21.5

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COMPARATIVE STUDY FOR ESTABLISHING SOME GENETIC MARKERS IN THE AUTOCHTHONOUS CARP BREEDS "FRĂSINET" AND "ROPŞA" (*CYPRINUS CARPIO*, L.)

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The cytogenetic analysis and comparative study of the muscle proteins were carried out in the "Frăsinet" and "Ropşa" carp in order to establish the genetic markers in the adult populations, in the direct descendants and in the ones obtained by reciprocal crossing.

The fishery sector in stations and basins undertakes to investigate the acclimatization of some foreign fish species and the breeding of the autochthonous ones by creating new breeds and varieties, which should better turn into profit the natural food resources and should also reduce to a minimum the supplementary food.

The modern methods applied at present in scientific breeding are based on the thorough knowledge of morphological, physiological, biochemical and ethological characteristics of each individual, in a strong correlation with the genetic features of the studied forms to the one of genitors.

The determination of some differences by a careful study of chromosome morphology and the comparison of karyotypes with the forms of a genus or of a species, coupled to the phenotypical studies and to those of adaptation and superior specialisation of some lines or ameliorated forms have lately received an important part in establishing the results of selection.

The characterisation of the newly obtained or ameliorated forms claims a complex synoptical table including the knowledge of quantitative and qualitative morphological characters, both macroscopic and micoscopic ones, of biochemical properties and also the knowledge of the way of transmitting them to the descendants, of the necessary conditions in maintaining the useful genes and the elimination of economically unfavourable genes.

Generally, in the characterisation of the new forms in the carp, the breeders have specially followed the exterior aspect and certain plastic characters. Important is the case of intra- and interspecific crossbreedings to maintain some certain characters in the generations I, II, III, that is their constant maintaining in the genome.

The biochemical and genetical studies regarding the above mentioned aspects prove to be very useful; the literature reveals the value of such studies for selection. Thus, the characterisation of different selected lines of fish is possible using molecular markers consisting of molecules of the structural and enzymatic proteins made evident by the electrophoretic method.

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MATERIAL AND METHODS

The specimens used for the cytogenetic and electrophoretic analyses were collected from the experimental basins of the fishery research station of Nucet.

The method used for the study of chromosomes was as follows: the animals were colchicinized four hours before killing and kidneys were removed. Hypotonization was performed in sodium citrate solution (0.75%) for 45 minutes, at room temperature. After 4-5 successive fixations in ethanol/acetic acid solution (3:1) for 5 minutes each, followed by centrifugation at 1500 r.p.m. for 7 minutes, the preparations were made by the air drying-flame method and stained with Giemsa solution. The metaphases corresponding to the two forms ("Frăsinet" and "Ropşa") were examined and from the best ones karyotypes were made up.

For electrophoretic analysis, the skeletal muscles were dissected from each individual. The individual specimens were introduced into 0.05 M disodic phosphate buffer at pH 7, mechanically homogenised and centrifuged at 5000 r.p.m. at 0˚C for 15 minutes.

Vertical polyacrylamide gel of the supernatant was accomplished (Davis, 1994) in discontinuous buffer system following the Ornstein method (1964).

The gels were stained with Amidoschwartz 10 D 1% in 7.5% acetic acid for 6-7 hours.

The results are represented by schematic drawings of the obtained pattern bands[1].

RESULTS AND DISCUSSION

The history of cytogenetics in the genus Cyprinus begins with Makino's research (1939), who investigated the common carp and the mirror carp by the successive fraction technique.

The investigations continued with the studies of Ojima et al. (1966),Ohno and Atkin (1966), Ohno et al.(1967), Raicu and Taisescu (1972).

The comparative study performed by us in the "Frăsinet" carp and "Ropşa" .carp, have shown in both cases 2n=104 somatic chromosomes (Figs 1, 2, 3, 4).

The 104 chromosomes forming 52 de homologues consist of 3 groups of morphologies, as follows: Group A -12 pairs of metacentrics (M);- Group B - 12 pairs of submetacentrics, subtelocentrics (Sm, St);- Group C - 28 pairs of acrocentrics (A).

The numerical differences which appear in relation to other authors result from the phenomenon of polymorphism that frequently occurs with fish; in our case we talk about an interpopulation polymorphism.

Another cause which determines the appearance of a different number of chromosomes is the aneuploidia.

[1]The electrophoretic analyses were performed in the Molecular Biology Department of the Faculty of Biology, Bucharest

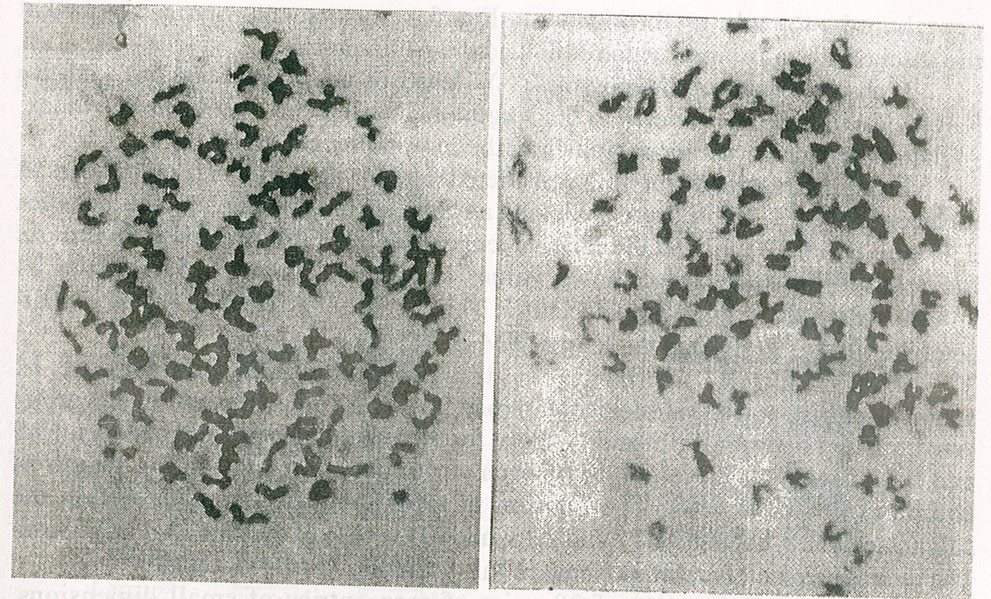

Fig.1 Fig. 2

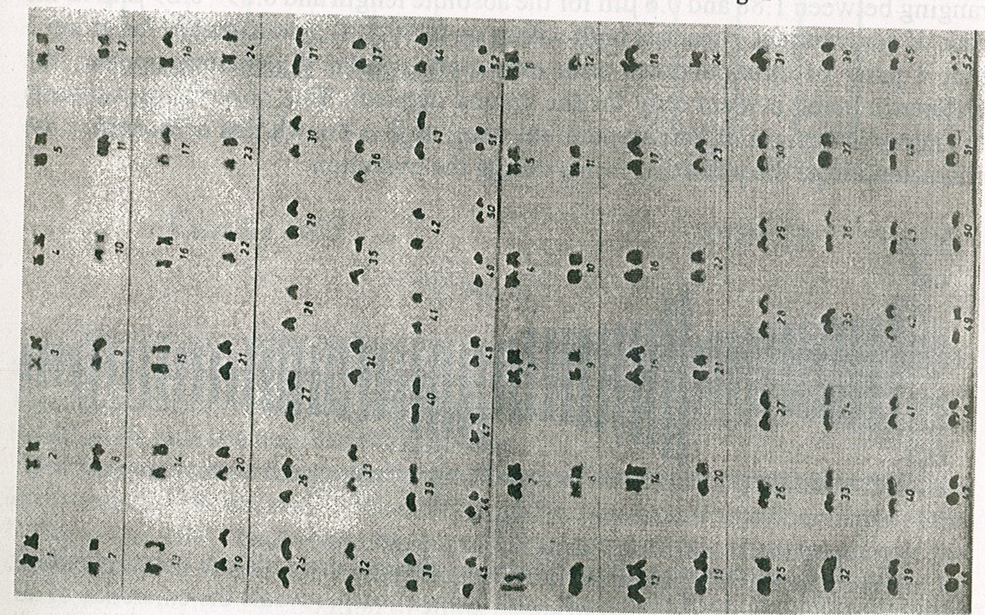

Fig. 3 Fig. 4

Thus authors like Szollar and Hobor (1972), in the special case *Cyprinus carpio*, present the average number of chromosomes as being $98^{\pm\frac{12}{6}}$; the same authors mention the low mitotic index (4-5% in liver, 3% in kidneys), which together with the large number of chromosomes, make difficult the statistic computation on the basis of biometrical measurement.

In order to make up the ideogram, Fig. 5, measurements were performed at the level of chromosomes which followed the parameters:

• absolute length of chromosomes (%); - length of arms - long, short (%); - relative length of chromosomes related to the length of complement; - centrometric index; - relation between arms for establishing the morphological group.

From the resulted data, it is considered that Group A consists of 12 pairs of metacentric (1-12) chromosomes, having an absolute length which ranges between 2.42 µm, and 1.22 µm, and the relative length between 11.8 µm and 0.56 µm. As the relation between arms shows values between 1.02 and 1.57, all the 24 chromosomes are defined as metacentric. The average chromosome which is characteristic of this group has the length $\overline{X}M$ = 1.85µm.

The Group B, according to the measurements, consists of 11 submetacentrics and one subtelocentric (pair 23). The limit value within this group is to be found between 3 µm and 1.32 µm for the absolute length and between 13.9 and 0.61 for the relative length of each chromosome related to the length of complement (21.47 µm). The relation of arms long/short, ranges within the submetacentrics, between 1.72 and 2.15, and in the case of the pair 23 it is 3.58µm. The average value of a chromosome in this group is $\overline{X}Sm$ = 2.25µm.

The group C consists of 28 pairs of acrocentrics of small dimensions, ranging between 1.88 and 0.6 µm for the absolute length and 0.89 - 0.29 µm. In the case of acrocentrics, *r* reaches high values up to 30.8. It is remarkable for group 3 that 4 pairs of chromosomes with extremely similar dimensions appear, the difference being evident only for the second decimal. This observation supports the tetraploid origin of karyotype in the carp, as it is known that acrocentrics are characteristic of the primitive forms during the evolution.

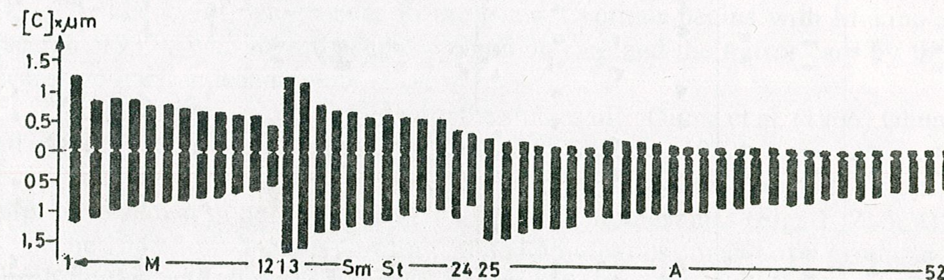

Fig.5

The study of proteic polymorphism of skeletal musculature (sarcoplasmic) within the populations of "Frăsinet" and "Ropşa" carp, reveals the existence of an intrapopulation variation, differences between the lines "Frăsinet" and "Ropşa" and also between the hybrid descendances ("Frăsinet" × "Ropşa", "Ropşa" × "Frăsinet").

In order to be able to compare the electrophoregrams we considered the "Frăsinet" race as a basic type, where males and females (sires) were studied. We proceeded in the same way with the "Ropşa" line (Fig. 6).

The obtained results show a striking similitude between the two lines for most of separated proteinic fractions. From this point of view, common proteinic fractions are those indicated with 1, 2, 3, 3a, and 10 in electrophoregrams.

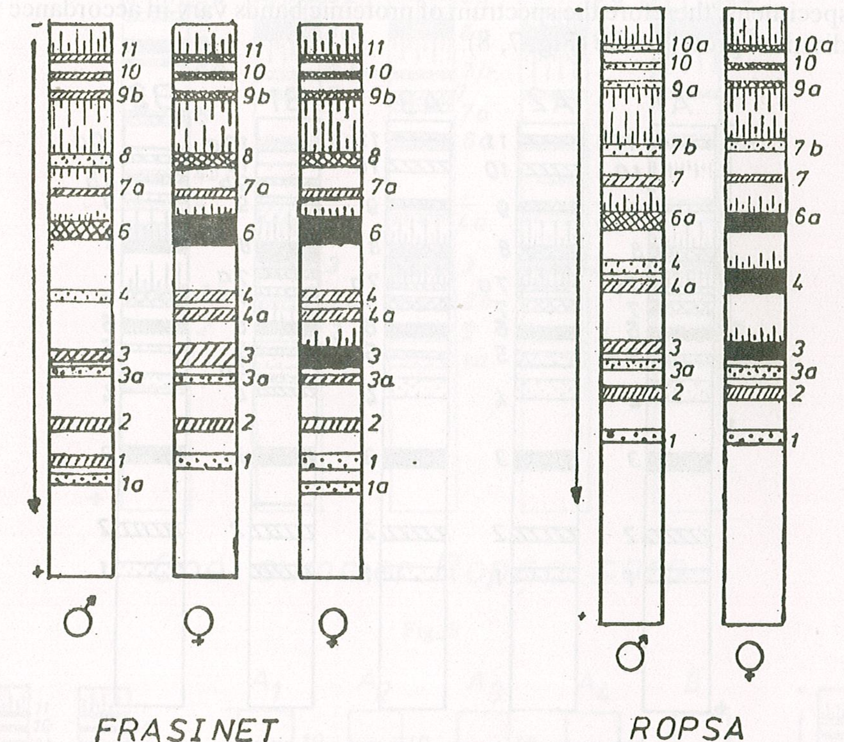

Fig. 6

The two lines differ by fractions 1a and 7 which in the "Frăsinet" carp appear as subfraction 7a, and in the "Ropşa" carp as 7b, with different positions in the gel. The fractions 1a, 8, 9b and 11 characterise only the "Frăsinet" line, and the fractions 9a and 10a only the "Ropşa" line.

Similarly, it is found that in the "Frăsinet" line some differences between sexes occur by the absence in the male of subfraction 4a and by colour depth of the bands in the gels - the females have a much higher proteinic concentration of the bands 3, 6, 8, 9,10 and 11; in the "Ropşa" line the differences are represented by the higher protein concentration of some bands in the female.

The comparative study of muscle proteins in the "Frăsinet" carp determined us to use the same analyses in the direct respective descendants - one-summer specimens - in order to clear up some aspects related to the ontogenetic phases of the synthesis of muscle proteins.

• As a first remark as regards one-summer specimens we find two types A and B in the "Frăsinet" carp and 3 types A, B and C in the "Ropşa" carp.

• The absence of major band 5 in the "Ropşa" type and the presence of subfractions 2b, 3a, 4b, 6a and 7b characteristic of this form are noticed.

• The one-summer specimens show, as it results from the gels analysis, proteic polymorphism within population as well as differences in relation to the adult specimens, therefore the spectrum of proteinic bands vary in accordance with the individual growth state (Fig. 7, 8).

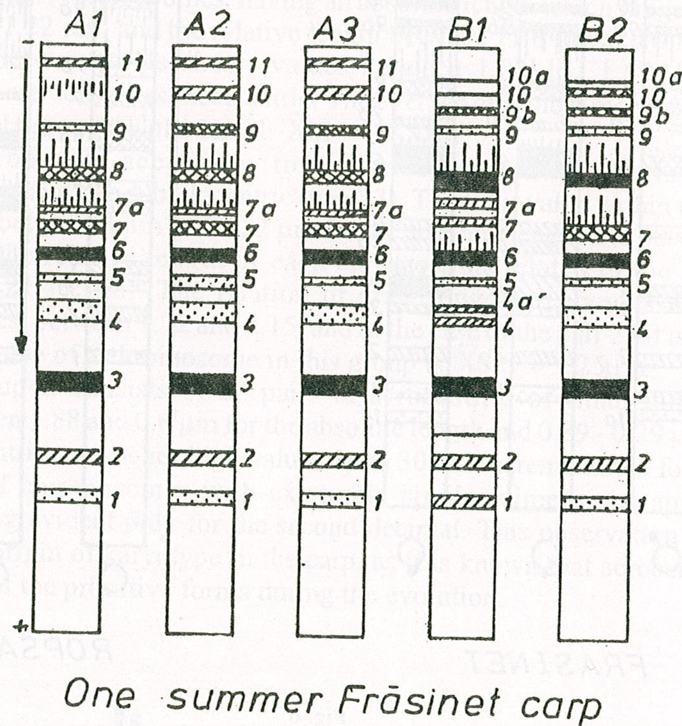

Fig. 7

After the analyses of the electrophoregrams of descendance resulted from the crossing between the "Frăsinet" female and "Ropşa" male, the presence of proteinic fractions of one or the other parent has been observed. The proteinic fraction 1a, present in all electrophoregrams is of a paternal origin, the maternal band 1 is absent (Fig. 9).

The fraction 2 is characteristic of both parents and the major fractions 3, 4, 5 and 6 are typically maternal both in width and colour intensity. Unlike the genitors in the major fraction 4 in electrophoregrams of hybrids no subfractions are present, and band 5, present in hybrids, is absent in type B.

The fraction 7 is common to the species and therefore present in the electrophoregrams of hybrids, like the subfraction 7a, characteristic of the maternal genitor.

An obvious difference between the hybrids and both genitors is the fraction 8, which is absent in electrophoregrams of type A; and present only in type B. The type B is of a maternal origin as regards the intensity and diffusion.

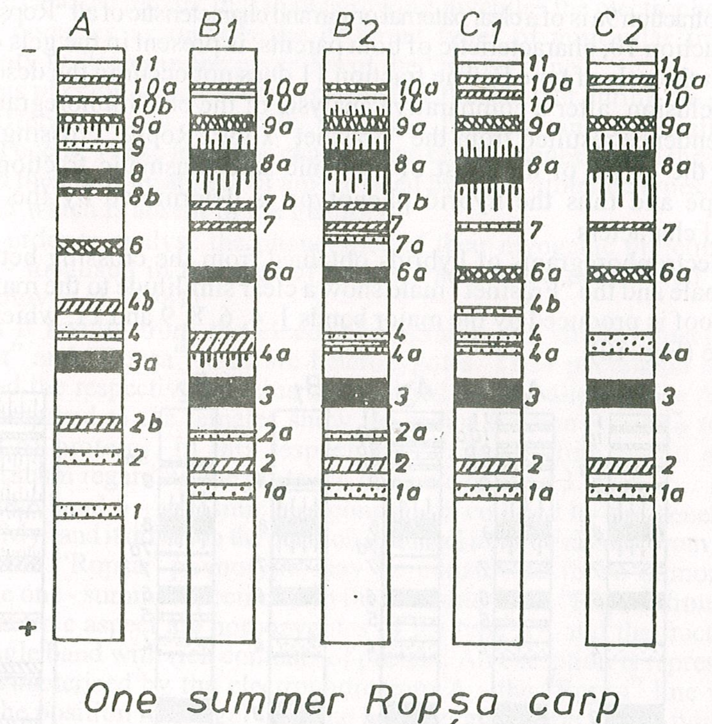

Fig. 8

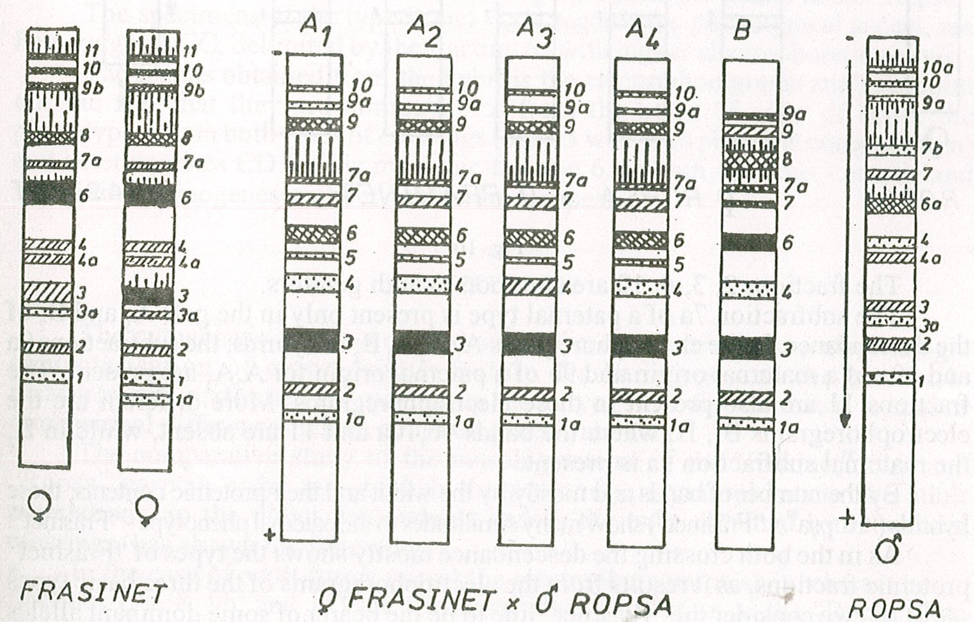

Fig. 9

The subfraction 9a is of a clear paternal origin and characteristic of all "Ropşa" breeds.

The fraction 10, characteristic of both parents, is present in the gels of type A and absent in the gels of type B. The fraction 11 does not occur in the descendants.

In conclusion, after a comparative analysis of the electrophoregrams of the hybrid descendence resulted from the "Frăsinet" x the "Ropşa" crossing, one can remark that the origin of the most of proteinic sarcoplasmatic fractions is of a maternal type and thus the hybrid phenotype is determined by the maternal phenotypical characters.

The electrophoregrams of hybrids obtained from the crossing between the "Ropşa" female and the "Frăsinet" male show a clear similitude to the male phenotype. The proof is produced by the major bands 1, 4, 6, 8, 9 and 11, which are of a paternal type (Fig. 10).

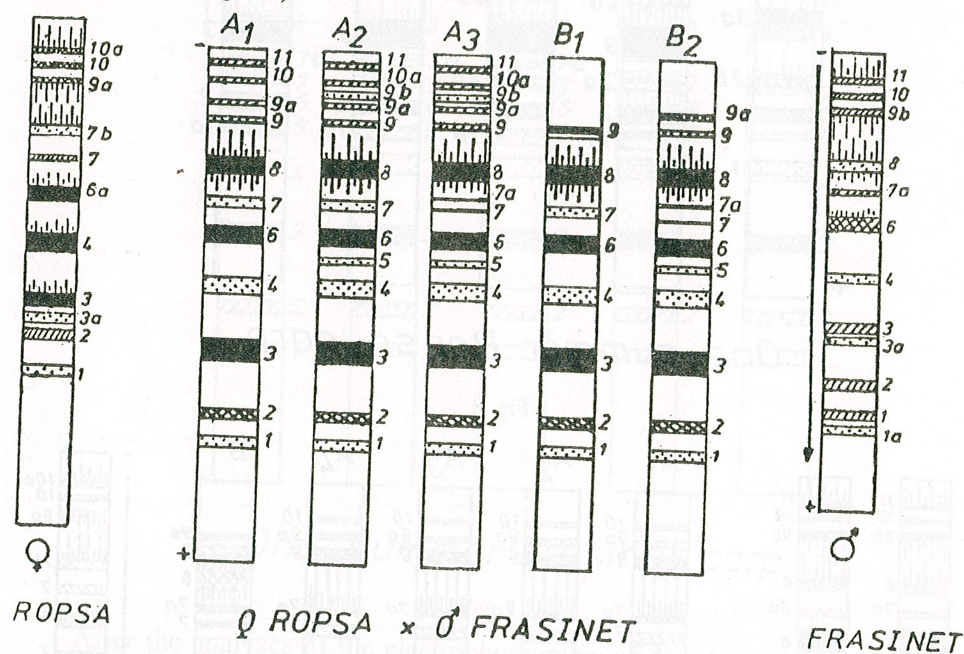

Fig. 10

The fractions 2, 3, 6, 10 are common to both genitors.

The subfraction 7a of a paternal type is present only in the gels A_3 and B_2 of the descendance. In the electrophoregrams A_1 A_2 A_3 B_2 in hybrids, the subfractions 9a and 10a of a maternal origin and 9b of a paternal origin for A_2A_3 are present. The fractions 11 are also present in these electrophoregrams. More different are the electrophoregrams B_1, B_2 where the bands 9b,10a and 11 are absent, while in B_2 the maternal subfraction 9a is present.

By the number of bands and mostly by the width and their proteinic contents, these hybrids ("Ropşa" x "Frăsinet") show many similitudes to the paternal phenotype - "Frăsinet".

As in the both crossing the descendance mostly shows the types of "Frăsinet" proteinic fractions, as it results from the electrophoregrams of the three basic types -A, B, C- we consider the "Frăsinet" line to be the bearer of some dominant alleles in relation to the "Ropşa" line.

We can also remark that in the growing juveniles the electrophoretic Table 4 is more complex as regards the different types of molecular fractions. This complexity is clearer in the juveniles of the "Ropşa" line.

This phenomenon may be explained by the fact that during the growth stage, in its different phases, generally proteinic fractions and especially enzymatic fractions occur. These fractions characterise certain stages where they play precise parts and then they disappear or are replaced by other proteinic types (for example fraction 5 which is absent in the genitors).

In order to analyse the phenotypes of their myogenic proteins – actin and myosin - localised in the area of bands 3 and 6, we considered the paper of Dobrovolov et al. (1981).

As it results from the electrophoregrams, the studied specimens of the "Frăsinet" and "Ropşa" lines are heterozygotes. Their phenotype is of the type "Aa", and the respective proteins are codified by two allele genes Ac^A, Ac^a. The males compared to the females show the major fraction 3 with a more reduced contents of proteins. In this respect the "Frăsinet" line shows a more clear polymorphism regarding the proteinic contents of band 3.

In the band 6 - myosinic component - is codified by the genes of the type My^C and My^D and it differs in the position in the gel in the "Frăsinet" from the "Ropşa".

In the "Ropşa" phenotype may be considered to be homozygotic CC.

The one - summer specimens in the "Frăsinet" and "Ropşa" from the point of view of actinic aspect are homozygotes of the type AA and the fraction 3 occurs like a single band with rich contents of protein. An exception is represented by the group characterised by the electrophoregram A - the "Ropşa" line where 3a, as regards the position in the gel is of the heterozygotic type, of the type Aa.

The myosinic fraction (band 6) in the "Frăsinet" occurs as a heterozygote of the type CD and this situation is the same in the specimens of the type A in the "Ropşa".

The specimens of the type B and C, as regards the phenotypical aspect, are homozygotes CC, delimited by the fraction 6a with higher electrophoretic activity.

The results obtained from the hybrids the electrophoregrams analysis point out the fact that they are homozygotes from the point of view of actin, the phenotype AA, in both types of crossings (band 3 with high proteinic concentration) and heterozygotes CD for the myosinic fraction 6. In both fractions - actinic and myosinic - homogenes without subfractions appear.

CONCLUSIONS

The cytogenetic analysis carried out in the carp forms by the morphologic characterisation of chromosomes within the kariotype and the biometric measurements performed for making up the karyograms did not reveal formal or dimensional differences which could be genetic markers.

The comparative study of the muscle proteins in the "Frăsinet" carp and "Ropşa" carp in order to establish the proteinic polymorphism in the adult populations, in the direct descendants and in the ones obtained by reciprocal crossings, has shown the following:

- - In the case of adult forms (sires) the electrophoregrams show characteristic fractions (7a, 8, 9b, and 11) for "Frăsinet" lines in relation to the "Ropşa" line (fractions 7b, 9a,10b);

• - Clear proteinic sexual dimorphism is observed by the colour intensity of the bands and by the absence of the subfraction 4a in the "Frăsinet" males; the "Ropşa" carp is characterised by a higher protein concentration of certain bands (3, 4, 6a,10,10a) and by the absence of the subfraction 4a in the females;
• - The direct descendants, the one-summer specimens, respectively, show as it is observed after the analysis of gels, both intrapopulation proteinic polymorphism (2 types A and B in the "Frăsinet", types A, B, C in the "Ropşa") and differences in relation to the adult specimens, therefore the spectrum of bands varies in accordance with the individual growth - stage;
• - In the case of descendants resulted by reciprocal crossings between the "Frăsinet" × "Ropşa" and "Ropşa" × "Frăsinet" clear is the preponderant way of transmitting the "Frăsinet" fractions which may be considered as the bearer of dominant alleles;
• - The phenotype analysis of the myogenic proteins - actin and myosin - localised in the area of bands 3 and 6, led to the establishing of the following phenotypes:
§ The heterozygote adult "Frăsinet" individuals for the actin $Ac^A Ac^a$ and for myosin $My^C My^D$; The "Ropşa" adults are heterozygotes $Ac^A Ac^a$ and homozygotes $My^C My^C$;
§ The one - summer "Frăsinet" specimens are phenotypes $Ac^A Ac^A$ and $My^C My^D$; the "Ropşa" specimens, at the same age, are $Ac^A Ac^A$ for actin, and myosin the type A is $My^C My^D$, and the types B and C are $My^C My^C$;
§ The descendants of both crossings are heterozygote for actin and myosin - $Ac^A Ac^a$ and $My^C My^D$ respectively.

In electrophoregram bands, characteristic of different lines, occur which subsequently can be followed in the direct or hybrid descendants.

The data obtained allow us to conclude that the electrophoretic analysis of sarcoplasmatic proteins makes possible their use in investigating the evolution of certain fish lines during their acclimatisation and selection processes.

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La «Revue roumaine de biologie - Série de biologie animale» publie des articles originaux d'un haut niveau scientifique de tous les domaines de la biologie animale : taxonomie, morphologie, physiologie, génétique, écologie, etc. Les sommaires des revues sont complétés par d'autres rubriques, comme: 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie: symposiums, conférences, etc.; 2. Comptes rendus des livres de spécialité.

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

Les tableaux et les illustrations seront numérotés en chiffres arabes.

La répétition des mêmes données dans le texte, les tableaux et dans les graphiques sera évitée. Les références bibliographiques citées par ordre alphabétique comporteront le nom et l'initiale du prénom de l'auteur, l'année, le titre de la revue abrégé conformément aux usances internationales, le tome, le numéro, la page.

Les travaux seront accompagnés d'un court résumé de 10 lignes au maximum, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages (y compris les tableaux, la bibliographie et l'explication des figures).

La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.