Toute commande sera adressée à:
EDITURA ACADEMIEI ROMÂNE, Calea 13 Septembrie nr. 13, Sector 5, P.O. Box 5–42, Bucureşti, România, Tel. 401-411 9008, Tel./Fax. 401-410 3983, 401-410 3448; e-mail: edacad@ear.ro
RODIPET S.A., Piaţa Presei Libere nr. 1, Sector 1, P.O. Box 35-37, Bucureşti, România, Tel. 401-618 5103; 401-222 4126, Fax 401-222 64 07.
ORION PRESS IMPEX 2000, P.O. Box 77-19, Bucureşti 3, România, Tel. 401 – 653 7985, Fax 401-324 0638.

Les manuscrits ainsi que toute correspondance seront envoyés à la rédaction; les livres et les publications proposés en échange seront adressés à: INSTITUTUL DE BIOLOGIE, Splaiul Independenţei 296, P.O. Box 56-53, 79651, Bucureşti.

REVUE ROUMAINE DE BIOLOGIE
SÉRIE DE BIOLOGIE ANIMALE
Calea Victoriei 125
R-79 717, Bucureşti, România
Tél. 650 76 80

REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE BIOLOGIE ANIMALE

TOME 44 N° 2 Juillet-Décembre, 1999

SOMMAIRE

MARIN FALCĂ, Numerical structure of Lumbricidae populations from the soils of some beech-fire mixed forests from the Bucegi National Park .. 113

AURA LUNGU, MARIANA ZGLOBIU, OTILIA MIHAIL, Dynamique des communautés des planctons du lac d'accumulation Budeasa 121

LILIANA VASILIU-OROMULU, La distribution altitudinale des espèces de Thysanoptères (Insecta: Thysanoptera) du Massif Gârbova 131

C. SEVCENCU, A. PATRICIU, C. ARDELEAN, Designing and using of a data acquisition system for parallel monitoring of both bioelectrical and mechanical activities of isolated frog heart 139

Al.G. MARINESCU, DANA RICHTER, DIANA DINU, Ş. ANDREUȚĂ, A. MARINESCU, Recherches sur l'utilisation de l'activité transaminasique (GOT) dans l'évolution de la qualité du sperme de verrat par rapport au régime de réfrigération et de congélation 151

OTILIA ZĂRNESCU, Incorporation of vitellin-gold by ovarian follicles of *Carassius auratus gibelio* II. *In vivo* studies..... 159

PAULA PRUNESCU, Hematopoietic bone marrow in the experimentally lead poisoned rats..... 165

VIORICA MANOLACHE, VIRGINIA POPESCU-MARINESCU, MARIA NĂSTĂSESCU, DANIELA TEODORESCU, ELENA NEAGU, Structural changes caused by lead action on the hepatopancreas of *Anodonta cygnaea* L. (Mollusca, Lamellibranchiata) 175

VIRGINIA POPESCU-MARINESCU, MARIA NĂSTĂSESCU, VIORICA MANOLACHE, DANIELA TEODORESCU, ELENA NEAGU, Structural changes caused by lead action on gills of *Anodonta cygnaea* L. (Mollusca, Lamellibranchiata) 181

REV. ROUM. BIOL.–BIOL. ANIM., TOME 44, N° 2, P. 111–214, BUCHAREST, 1999

MARIA NĂSTĂSESCU, VIORICA MANOLACHE, VIRGINIA POPESCU-MARINESCU, DANIELA TEODORESCU, ELENA NEAGU, Structural changes caused by lead action at the level of *Anodonta cygnaea* L. (Mollusca, Lamellibranchiata) mantle 189
CONSTANTIN DELCEA, Bacterial denitrification of municipal wastewater at Bucharest-Glina purification plant 197
AL SAKKAF GALAL, IRINA TEODORESCU, Amoebiasis correlated with socio-economic factors in two areas of Yemen 203
MARIOARA FINTA-ISTRATE, ANCA VOICU, I. LAZĂR, The determination of irritation potential of biopolymer of *Xanthan* type on rabbits 209

NUMERICAL STRUCTURE OF LUMBRICIDAE POPULATIONS FROM THE SOILS OF SOME BEECH-FIRE MIXED FORESTS FROM THE BUCEGI NATIONAL PARK

MARIN FALCĂ

Species composition, numerical density and relative abundance of earthworm populations in the soils of beech-fire mixed forests from the Bucegi National Park have been established. It is emphasised that there are differences between those three forests that were studied, as far as the parameters described above are taken into consideration.

1. INTRODUCTION

The Bucegi National Park comprises an area of 55105 ha (the area is different in other studies), from which 33680 ha represent the forestry area and 21425 ha represent alpine pasture, low pasture, arable meadow and county areas. Beech-fire forests with mull flora are characteristic of the middle mountain and occupy 4133 ha in the area of the Bucegi National Park. Among soil animal groups, earthworms represent a biocenotical component with an important role in decomposition and mineralization of organic material. Species composition, numerical density and relative abundance of earthworm species from three areas with fire and beach mixed forests on the Prahovean Part of the Bucegi Mountains are emphasised in the present paper.

2. METHODS AND WORKING TECHNICS

On the Prahovean Slope of the Bucegi Mountains, in the area of Sinaia-Buşteni-Azuga, 3 representative research points with beech-fire mixed forests were established as follows:

1. *Zgarbura Valley*, 990–1100 m altitude, East, North-East exposure, with a slope of 10 - 250; the soil is forestry mountain brown, formed on Sinaia deposits; characteristic mull flora with *Carex sylvatica*.

2. *Peleş Valley*, 1000–1050 m altitude, East, North-East exposure, 100 slope, forestry mountain brown soil, formed on Sinaia deposits, characteristic mull flora with *Oxallis acetosella*.

3. *Boncu – Buşteni*, 1050–1200 m altitude, South, South-East exposure, 10–15°, forestry brown mountain soil, formed on Sinaia deposits, mull flora with *Oxalis acetosella*.

Faunistic material was monthly collected, from June to October, using a metallic frame 25/25 cm long. Earthworm individuals were extracted by hand from soil samples.

REV. ROUM. BIOL.–BIOL.ANIM., TOME 44, N° 2, P. 113–120, BUCAREST, 1999

3. OBTAINED RESULTS

Species composition of earthworms comprises 14 species: *Aporrectodea rosea rosea, Aporrectodea caliginosa caliginosa, Allolobophora georgii, Allolobophora dacica, Lumbricus terrestris, L. improvisus, L. sp., Eisenia lucens, Octolasion lacteum, Octolasion sp., Octodrilus lissaensis, Dendrodrilus rubidus rubidus, Dendrobaena octaedra, Dendrobaena alpina alpina.* From these species, some are specific for beech forests, as *Aporrectodea rosea rosea, A. caliginosa caliginosa, A. dacica, A. georgii, O. lacteum, L. terrestris*; some others are frequent in fire forests, generally in the upper level of vegetation as *Dendrobaena alpina alpina, D. octaedra, Dendrodrilus rubidus rubidus*; the other ones are more frequent in the mixed beech and fire forests as *Eisenia lucens*. In all three searched areas, from all 14 identified species, a number of 3, representing 21%, were identified in all areas; 5 species, representing 36%, were identified in the fire-beech mixed forest from Valea Zgarbura; 3 species, representing 21%, were identified only in the area of Peleş Valley; 3 species, representing 21%, were identified in the Boncu-Buşteni area. These data show some aspects, which corroborating with some other soil animal groups and with vegetation data, are defining much better structural peculiarities of mixed fire-beech forests. Analysing percentages through which common species in all 3 searched areas are represented, one conclusion may be established which underlines the idea that the number of common species is small, taking into account that the structural parameters of soil and vegetation are defining a distinct forestry unit, mixed fire and beech forest, different from fire and beech ones. A small number of common earthworm species indicates three types of forests, with a near similar species composition, rather a distinct one of mixed fire and beech forest. Extending considerations on this aspect, by taking into account only the earthworm populations, one may conclude that mixed fire and beech searched forests in the Bucegi Mountains are less individualised as forestry type, as compared with beech and fire forests.

Numerical density has been put into evidence through the individual average numbers on square meter and is shown in Table 1. Analysing this table, there are differences between those three stations, both as the individual average number on square meter and as its monthly dynamics. Thus, *Aporrectodea rosea rosea*, showing the highest numerical density in all three areas, registers those three areas on a curve with decreasing values from Zgarbura Valley to Boncu-Buşteni and Peleş Valley.

Vertical distribution of the individuals is nearly uniform from top to 30 cm soil depth, at Peleş and Zgarbura Valleys, with a numerical peak of juveniles at the level of 10 cm soil depth at Boncu-Buşteni.

Juvenils/adults ratio is almost maintained at supraunit values, without maintaining a constant character in all stations and at all depth levels. This idea is illustrated by *Aporrectodea rosea rosea* species at Boncu-Buşteni station, where juvenils are three times higher than adults, at 10 cm soil depth; at 20 cm soil depth, adults are nearly two times higher than juvenils. Adults of earthworms, depending on the place where they feed, are epigeic, endogeic and anecic species. That means that earthworm species do have functional peculiarities, as far as feeding habit is concerned. Juvenils, not taking into account small vertical movements, are usually found at the same depth level as adults are. Because of that, there are no more informations on the adults/juvenils ratio at the same depth level, than those on the developmental potential of the respective populations. The subunit adults/juvenils ratio at 10 cm soil depth, at Boncu-Buşteni, and the supraunit ratio at 20 cm soil depth, in the same station, shows a higher numerical potential at the first soil level, as compared with the second soil level.

It is to notice the lack of species of *Dendrobaena* genus at Boncu-Buşteni, where, excepting June, relative humidity of soil was slower, as compared with other two stations.

Relative abundance (Table 2) shows the place of each species in the spectrum of all earthworm populations and it emphasises *Aporrectodea rosea rosea* and *Octolasion lacteum* with the highest values, in all stations. These are ubiquist species, abundant in all soils, the only ones which form the group of edified species. These two species represent 25% of all earthworm species at Zgarbura and Peleş Valleys and 33% at Boncu-Buşteni.

As far as the number of individuals of those two species is concerned, they represent 90% at Zgarbura, with the mention that the individuals of *Aporrectodea rosea rosea* represent 80% and only 10% for *Octolasion lacteum*, in June. For all months in which observations were made, in June, August, September and October, the number of individuals of those two species represents 60%. That is because of higher individual numbers of *Dendrodrilus rubidus rubidus* and *Octodrilus lissaensis*, in August. At Peleş Valley, those two species are represented with a 25%, taking into account the number of all earthworm species identified in this station, and with 49%, taking into account the number of individuals of all species. At Boncu-Buşteni, those two species are represented with 33%, taking into account the number of all earthworm species, and with 100%, taking into account the number of all earthworm species, and with 100%, taking into account the number of all individuals in June, July and August, 80% in September and 78% in October (Table 3). These percents illustrate the dominance of those two species, the other ones being accompanying ones.

Table 1

Numerical density of earthworm species (average individual number/m^{-2})

Species	Level	Stadium	Zgarbura Valley					Peleş Valley					Boncu-Buşeni				
			June	July	August	Sept.	Oct.	June	July	August	Sept.	Oct.	June	July	August	Sept.	Oct.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Aporrectodea rosea	L	juv.	6.86								6.86		4.57		4.57	22.86	4.57
	S_1	juv.	6.86		9.15						2.29	2.29				6.86	2.29
		ad.	6.86		2.29						9.15					9.15	
	S_2	juv.	4.57		6.86	6.86					4.58	2.29	2.29			16.00	2.29
		ad.	6.86		9.15	4.58											
	S_3	juv.			13.71												
		ad.	4.57		2.29												
Aporrectodea caliginosa	S_2	ad.				2.29											2.29
Allolobophora georgii	S_1	ad.									2.29						
Allolobophora dacica	S_2	ad.							4.57							2.29	
Lumbricus terrestris	L								11.4	11.4	2.29					6.86	
	S_1	Juv.							6.86		11.4						
	S_2	juv.								9.15					2.29		
Lumbricus improvisus	S_1	juv.								2.29							
		ad.			2.29					2.29							
	S_2	juv.								2.29							
	S_3	juv.															

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Lumbricus sp.	S_1	juv.							6.86								
Eisenia lucens	S_2	ad.															2.29
Octolasion lacteum	L	juv.						2.29					2.29		2.29		
		ad.	2.29						13.7						2.29		
	S_1	juv.								2.29			4.57	2.29	4.57		2.29
		ad.	2.29		4.57			6.86	2.29	20.5			9.15	2.29	2.29		2.29
	S_2	juv.							2.29			2.29					6.86
		ad.					4.57		2.29			2.29	6.86	2.29	9.15		
	S_3	juv.								2.29							
		ad.			4.57				2.29	4.57				4.57	4.57		
Octolasion sp.	S_1	juv.	2.29	9.14													
		ad.		9.14													
	S_2	ad.		6.86													
	S_3	ad.		6.86													
Octodrilus lissaensis	S_2	juv.			9.15												2.29
		ad.			2.29												
	S_3	juv.			9.15												
		ad.			2.29												
Dendrodrilus rubidus	S_1	juv.			4.57							2.29					
		ad.			2.29												
	S_2	juv.			2.29		2.29										
		ad.				2.29											
Dendrobaena octaedra	S_1	juv.								13.7							
		ad.							2.29								
	S_2	juv.										4.57					
	S_3	juv.								2.29							
Dendrobaena alpina	L	juv.	2.29														
	S_2	ad.				2.29											

Table 2

Relative abundance of earthworm species (%)

Species	Zgarbura Valley					Peleş Valley					Boncu-Bușteni				
	June	July	August	Sept.	Oct.	June	July	August	Sept.	Oct.	June	July	August	Sept.	Oct.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Aporrectodea rosea rosea	80		51	61					59	28	23		14	83	36
Aporrectodea caliginosa				13											
Allolobophora georgii															
Allolobophora dacica															
Lumbricus terrestris															
Lumbricus improvisus			3										6		10
Lumbricus sp.															
Eisenia lucens															
Octolasion	10		10		66	10				30	77	100	80		45
Octolasion sp.	5	100													9
Octodrilus lissaensis			26				13		6				6	17	
Dendrodrilus rubidus			10	13	34		42	16	35						
Dendrobaena octaedra								22							
Dendrobaena alpina	5			13		100	42	40		14					
							3	22							

Table 3

Peculiarities of edificated species group

Zgarbura Valley	Peleş Valley	Boncu-Bușteni
Species peculiarities	Species peculiarities	Species peculiarities
Aporrectodea rosea 25% from the number of individuals	*Aporrectodea rosea* 25% from the number of individuals	*Aporrectodea rosea* 33% from the number of individuals
Octolasion lacteum 60% from the number of individuals	*Octolasion lacteum* 49% from the number of individuals	*Octolasion lacteum* 86% from the number of individuals

4. CONCLUSIONS

Beech-fire mixed forests from the Zgarbura Valley, Peleş Valley and Boncu-Bușteni, located in the Bucegi Mountains, show some peculiarities which, at least from the point of view of species composition, form rather three distinct forests than a well definite forestry unit forestry, named beech-fire mixed forest. Thus, from the total number of 14 earthworm species identified in all three areas, only three (21%) were common for all three areas, with the highest species number at Zgarbura Valley (5 = 35%).

The highest numerical density showed *Aporrectodea rosea rosea* and *Octolasion lactem* species, which presented the highest values in all three stations. The same two species presented the highest percentages of relative abundance, being the only two species which formed an edificated group of species.

The slowest individual numbers presented *Aporrectodea caliginosa caliginosa*, *Allolobophora georgii* and *Allolobophora dacica*, with only one adult individual in all three stations.

REFERENCES

1. Beldie Al., *Flora şi vegetaţia Munţilor Bucegi*. Edit. Academiei RSR. Bucureşti, 1967.
2. Easton E. G., *A guide to the valid names of Lumbricidae* (Oligochaeta) in "Earthworm Ecology from Darwin to vermiculture". Ed. Chapman and Hall. 1983.
3. Falcă M. Oromulu Liliana. Honciuc Viorica. St. cerc. Biol., Seria biol. Anim., 1992, **44**, 2, 101–110.
4. Falcă M., Oltean M., Mitt.hamb.zool.Mus.Inst., 1994, **89**, 2, 83–87.

5. Falcă M., Argessis - St. şi com. Seria Şt. Naturii, 1995, **VIII**, 309–313.
6. Hendrix P. F., Mueller B., Bruce R., Langdale G. W., Parmelee R., Soil Biology and Biochemistry, 1992, **24**, *12*, 1357–1363.
7. Pop V., *Lumbricidele din România*, Edit. Academiei R.P.R., Bucureşti, 1948.

Received July 10, 1999

Institute of Biology,
Splaiul Independenţei 296
Bucharest 79651
P.O. Box 56 - 53
Romania

DYNAMIQUE DES COMMUNAUTÉS DES PLANCTONS DU LAC D'ACCUMULATION BUDEASA

AURA LUNGU*, MARIANA ZGLOBIU*, OTILIA MIHAIL*

Mots clefs: développement durable, équilibre écologique, biotope, biocénose, eutrophication.

Le développement durable de l'économie impose l'harmonisation des ressources nécessaires, avec les disponibilités existantes selon une stratégie générale, où la protection de la qualité des facteurs d'environnement occupe une place significative. Dans ce contexte on peut établir la capacité de support de la biosphère par rapport à l'impact sollicité par une société moderne.

L'eau en tant que facteur d' environnement et du développement socio-économique représente un domain très important pour la recherche scientifique en vue de résoudre les problèmes de conservation de la qualité des eaux ainsi que de maintenir les possibilités de l'utiliser en bon état.

1. INTRODUCTION

L'effet de l'action humaine sur l'environnement dépend du mode d'agression, de son intensité et aussi du niveau de supportabilité de l'écosystème sur lequel on applique l'action anthropique.

Par conséquence, l'évaluation et la prévention de l'impact humain sur les facteurs d'environnement impose la détermination des limite maximales jusqu' où l'on peut modifier les écosystèmes sans affecter visiblement leur intégralité.

Pour satisfaire les demandes d'eau qui augmentent sans cesse, l'alimentation, industrie, obtention de l'énergie électrique, irrigations etc. la régularisation des rivières a été nécessaire à cause de la construction des lacs d'accumulation et des canaux de fuite.

Sous l'action des facteurs anthropiques, dans les écosystèmes aquatiques apparaissent des modifications significatives des paramètres hydrologiques et physico-chimiques ayant des répercussions sur les paramètres biologiques qui modifient les relations biotope biocénose, la structure et la fonctionnalité des biocénoses ou bien troublent les équilibres écologiques.

Par la régularisation de la rivière Argeş on a construit en cascade une chaîne de lacs d'accumulation, le lac de Budeasa ayant une importance particulière grâce à son utilisation principale: l'alimentation en eau potable de la ville Piteşti.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 44, N° 2, P. 121–130, BUCAREST, 1999

2. MATÉRIAUX ET MÉTHODES DE TRAVAIL

Les investigations sur le terrain et celles faites dans les laboratoires pour analyser la qualité de l'eau de Budeasa du point de vue physico-chimique et biologique ont été effectuées pendant 1997, par saisons, dans des sections de contrôle représentatives -entrées dans le lac et le barrage, par profiles de profondeur (0m, 5 m, 10 m).

Les résultats obtenus ont été comparés avec les valeurs standardisées existantes: STAS 4706 –88 pour les eaux de surface:

– Guide méthodologique pour surveiller la qualité des eaux par des analyses biologiques (ICPGA, CNA, Bucarest 1984);

– Guide méthodologique pour surveiller l'évolution trophyque des lacs d'accumulation et des lacs naturels (ICIM, Bucarest, 1995).

Les déterminations taxonomiques ont été côntrolées par des spécialistes de l'Institut de Biologie – Académie Roumaine de Bucarest.

3. RÉSULTATS ET DISCUSSIONS

On ne peut présenter l'évolution des biocénoses caractéristiques du réservoir de Budeasa pendant 1997 sans une analyse préalable des principaux facteurs physico-chimiques de l'écosystème aquatique mentionné.

Les analyses physico-chimiques saisonnières faites pour l'eau du réservoir Budeasa comprennent un numéro relativement élevé d'indicateurs de qualité. On a analysé seulement les indicateurs qui puissent nous aider à déterminer la catégorie et les conditions de qualité pour les eaux de surface.

Par conséquence, en dehors de la température et la transparence de l'eau on a analysé aussi les indicateurs chimiques généraux c'est-à-dire le pH. le régime d'oxygène (oxygène dissous et CBO_5), l'apport des principaux nutriments (N et P).

Les résultats des analyses physico-chimiques, concernant l'évolution de la qualité de l'eau du réservoir de Budeasa, du point de vue chimique, pendant 1997, sont présentés dans le tableau 1.

Durant toute la période étudiée, les valeurs élevées d'oxygène dissous (9.3-16.84 mgO_2/l) indiquent que l'eau a été bien aérée; en été et en automne on a remarqué des sursaturations constantes en oxygène (115-184%) qui correspondent au stade (niveau) oligotrophe.

Les valeurs de la CBO_5 généralement sous 5.0 mgO_2/l, indiquent un chargement de l'eau pauvre en substances organiques biodégradables, exceptant l'été quand les organismes du plancton sont plus développés et la biomasse est plus nombreuse (élevée).

Les plus importantes substances biogènes sont le nitrogène et le phosphore qui, à côté de l'hydrogène et du carbone représentent les éléments principaux de la matière vivante; le phosphore qui fait partie de la structure des acides nucléiques a un rôle essentiel dans l'érédité des organismes.

Tableau 1

Les valeurs des indicateurs physico-chimiques
de l'eau du reservoir de BUDEASA pendant 1997

No. courant	Indicateurs physico-chimiques	U.M.	Saison	Sections de contrôle			
				entrée	barrage 0 m	barrage 5 m	barrage 10 m
1	température	°C	printemps	5,5	6	6	6
			été	22,5	22,5	20	20
			automne	16	17	17	16,5
2	transparence	cm	printemps		120		
			été		150		
			automne		150		
3	pH		printemps	7,2	7,3	7,4	7,4
			été	7,3	7,4	7,4	7,4
			automne	7,6	7,6	7,5	7,4
4	oxygène dissous	mgO_2/l	printemps	9,3	10,24	11,2	11
			été	16,84	16	13	11,2
			automne	11,8	13,2	11,3	11,2
5	saturation en oxigène	%	printemps	73,6	82,1	89,2	88,2
			été	180	184	144	124
			automne	120	137	118	115
6	CBO_5	mgO_2/l	printemps	0,4	0,7	0,4	1,8
			été	7,36	6,2	6,76	6,4
			automne	2,8	2,7	2,2	2
7	CCO-Mn	mgO_2/l	printemps	4,4	3,6	2,6	2
			été	8,8	7.5	7,4	6
			automne	4	4,4	3,6	3,8
8	CO_2 livre	mg/l	printemps	0	0	0	0
			été	0	0	0	0
			automne	1,1	1,1	1,1	1,1
9	HCO^-_3	mg/l	printemps	–	54,9	54,9	51,8
			été	85,4	73,2	73,2	79,3
			automne	42,7	42,7	42,7	48,8
10	CO^{2-}_3	mg/l	printemps	–	3	3	3
			été	12	6	12	12
			automne	0	0	0	0
11	N minéral total	mgN/l	printemps	1,38	0.86	0,48	0,54
			été	1.303	1,072	1,384	0,505
			automne	0,63	0,42	0,53	0,41
12	P total	mgP/l	printemps	0,32	0,6	0,078	0.039
			été	0,065	0.11	0,072	0,065
			automne	0,049	0,033	0,039	0,033

En outre, on peut observer dans le tableau 1 que les principaux nutriments du réservoir (N mineral total et P total) ont eu des concentrations entre 0,41–1.38 mg N/l, respectivement 0.0033–0.6 mg P/l; ces valeurs placent l'eau du réservoir de Budeasa dans la catégorie des eaux mésotrophes ayant une tendance d'eutrophication.

En conclusion on peut apprécier que les valeurs des principaux paramètres physico-chimiques de l'eau du lac d'accumulation de Budeasa pendant 1997 ont satisfait, sauf quelques exceptions, la première catégorie de qualité (STAS 4706-88) existante dans les années passées.

Connaître la structure de la flore et de la faune, donc la biodiversité de l'écosystème aquatique étudié, représente un critère très important pour apprécier l'état de qualité de celle-ci, pour constater en quelle mesure elle correspond aux utilisations pour lesquelles il a été construit.

L'analyse biologique de l'eau du réservoir de Budeasa a été effectuée parallèlement avec celle physico-chimique; on a prélevé les échantillons pour l'étude des principales biocénoses par saison et des mêmes sections de contrôle.

Pendant les recherches biologiques on a eu en vue l'évolution temporelle et spatielle des biocénoses caractéristiques (phytoplancton, zooplancton) tant du point de vue qualitatif – groupes systémiques, espèces, organismes indicatifs – que du point de vue quantitatif-densité, biomasse, abondance de la densité et de la biomasse –, le niveau de trophique des accumulations par suite des modifications résultées dans la structure du biotope sous l'influence des facteurs naturels et anthropiques.

Les résultats des analyses qualitatives et quantitatives sur la dynamique du phytoplancton du lac de Budeasa sont présentés dans les tableaux 2 et 3.

Le phytoplancton représente le principal producteur de matière organique vivante existante dans un ecosystème aquatique.

La biomasse phytoplanctonique, une mesure indirecte de la production primaire, peut fournir des données utiles pour évaluer le niveau trophique d'une accumulation. Dans la période froide de l'année par suite de la minéralisation des substances organiques, la concentration des éléments nutritifs de l'eau (azotates, azotites, phosphates) augmente et dans la saison chaude leur concentration baisse grâce à leur utilisation par les plantes (développement de la biomasse phytoplanctonique.

Du point de vue qualitatif pendant le printemps (avril) le phytoplancton du réservoir de Budeasa a été représenté par 8 taxons des groupes systématiques *Bacillariophyta, Euglenophyta et Chlorophyta*, les diatomées étant les éléments dominants qui ont représenté plus de 50% du total des organismes existants.

Parmi les algues identifiées représentées on mentionne: *Asterionella formosa, Cymbella compta, Cyclotella compta, Cyclotella meneghiniana, Cymbella sp.. Navicula sp.*, (diatomées); *Trachelomonas sp.* (euglenophytes); *Chlorella vulgaris, Crucigenia tetrapedia* (chlorophytes).

Tableau 2

Variation de la densité numérique (mil.cel/l) et l'abondance numérique de la densité (%) phytoplanctonique du lac d'accumulation de Budeasa – 1997

No. courant	Saison	Points de prélèvements	Densité totale mil.cel/l	Groupes systématiques									
				Cyanophyta		*Bacillariophyta*		*Pyrrophyta*		*Euglenophyta*		*Chlorophyta*	
				mil.cel/l	%	mil.cel/l	%	mil.cel/l	%	mil.cel/l	%	mil.cel/l	%
1	printemps	entrée	1,78	0	0	1,44	81,26	0	0	0,33	18,74	0	0
		barrage 0 m	3,57	0	0	1,86	72,23	0	0	0	0	0,71	27,77
		barrage 5 m	2,75	0	0	2,5	90,91	0	0	0,25	9,09	0	0
		barrage 10 m	2,11	0	0	2,11	100	0	0	0	0	0	0
2	été	entrée	0,7	0,02	3	0,54	77	0,06	9	0,014	1	0,07	10
		barrage 0 m	0,43	0	0	0,17	40	0,24	56	0,01	1	0,02	2
		barrage 5 m	0,42	0,01	1	0,15	36	0,23	55	0,02	2	0,03	7
		barrage 10 m	0,13	0,02	12,5	0,05	36,5	0,06	51	0	0	0	0
3	automne	entrée	5,14	0	0	5,14	100	0	0	0	0	0	0
		barrage 0 m	0	0	0	0	0	0	0	0	0	0	0
		barrage 5 m	1,7	0	0	1,1	67	0	0	0	0	0,57	33

Tableau 3

Variation de la biomasse (mg/l) et l'abondance de la biomasse (%) phytoplanctonique du lac de BUDEASA – 1997

No. courant	Saison	Points de prélèvements	Biomasse totale mg/l	Groupes systématiques									
				Cyanophyta		*Bacillariophyta*		*Pyrrophyta*		*Euglenophyta*		*Chlorophyta*	
				mg/l	%	mg/l	%	mg/l	%	mg/l	%	mg/l	%
1	printemps	entrée	3	0	0	1,7	56,67	0	0	1,3	43,33	0	0
		barrage 0 m	5,51	0	0	5,1	92,56	0	0	0	0	0,41	7,44
		barrage 5 m	6,4	0	0	5,4	84,38	0	0	1	15,62	0	0
		barrage 10 m	3,2	0	0	3,2	100	0	0	0	0	0	0
2	été	entrée	4,41	0,05	1	1,42	33	2,18	49	0,06	1	0,69	16
		barrage 0 m	13,36	0	0	0,97	7	12,36	92	0,016	0,5	0,013	0,5
		barrage 5 m	2,88	0,001	0	0,46	16	2,32	81	0	0	0,1	3
		barrage 10 m	3,5	0,001	0	0,52	14,5	2,97	85	0,008	0,5	0	0
3	automne	entrée	7,3	0	0	7,3	100	0	0	0	0	0	0
		barrage 0 m	0	0	0	0	0	0	0	0	0	0	0
		barrage 5 m	1,8	0	0	0,1	6	0	0	0	0	1,7	94

En été (juillet), dans la station de contrôle du barrage on a constaté une stratification de la cénose alguale d'une telle manière que la densité et la biomasse des algues ont été 3 – 4 fois plus grandes dans les profiles d'eau du 0 m et 5 m que dans celui de 10 m; cette situation peut être expliquée par la diminution de l'intensité de la lumière à cette profondeur.

Bien que les densités numériques des algues aient été relativement réduites, les valeurs de la biomasse phytoplanctonique de jusqu' à 13 mg/l (barrage 0 m) ont été dues à l'apport de *Ceratium hirundinella*, espèce ayant des dimensions grandes qui ont déterminé la floraison de l'eau du lac dans cette période.

En automne de 1997 (Octobre), le phytoplancton a été beaucoup plus pauvre et moins diversifié qu'en juillet. Les algues identifiées – diatomées et chlorphyicées ont été observées à l'entrée dans le lac et au barrage 5 m.

Parmi les phytoplanctons existants on mentionne: *Cymbella ventricosa, Fragilaria crotonensis, Nitzschia acicularis, Scenedesmus quadricauda*.

Sous l'aspect quantitatif, les valeurs de la densité et de la biomasse du phytoplancton du lac ont variée d'une saison à l'autre, en fonction des stations de contrôle et même des profiles de profondeurs.

Du point de vue du niveau trophique, le lac de Budeasa a été de type oligo-mesotrophe (la biomasse phytoplanctonique n'a pas dépassé 7.3 mg/l), ayant des tendances vers eutrophie (en été – 13 mg/l).

Le zooplancton représente l'anneau secondaire de la chaîne trophique dans un écosystème aquatique.

Tandis que la distribution du phytoplancton dans la masse de l'eau est limitée en général a l'épilimnion, la distribution et la fertilité des zooplanctons sont déterminées par l'abondance de la nourriture et le nombre des pilleurs.

Horne et Goldman affirment (1994) que les zooplanctons sont indépendants des bords ou du fond de l'eau. mais ils peuvent vivre à toutes profondeurs si l'oxygène existe dans des concentrations convenables.

La profondeur optimale pour la migration des zooplanctons est variable en fonction de saison. moment du jour. espèce, stage de vie et niveau trophique du lac.

L'évolution de la communauté du zooplancton de l'accumulation de Budeasa pendant 1997 este présentée dans le tableau 4.

L'analyse qualitative du zooplancton met en évidence la présence des rotifères. copépodes et claodocères pendant toute l'année et dans tous les points de contrôle. Au printemps. ont prédominé les stages jeunes de copépodes, en été, les cladocères ont été situées sur la première place.

Tableau 4

Dynamique du zooplancton -la densité (cx/l) et l'abondance (%) de la densité.
La biomasse (mg/l) et l'abondance de la biomasse (%) dans le lac BUDEASA 1997

No. courant	Saison	Points de prélèvements	Densité totale ex/l–%	Groupes systématiques			Biomasse	Groupes systématiques		
				Rotatoria ex/l–%	*Copepoda* ex/l–%	*Cladocera* ex/l–%	totale mg/l–%	*Rotatoria* mg/l–%	*Copepoda* mg/l–%	*Cladocera* mg/l–%
1	printemps	entrée	9	1-11,11	7-77,77	1-11,11	0,39	0,004-1,03	0,284-73,95	0,1-25,77
		barrage 0 m	32	2-6,25	24-75,0	6-18,75	1,37	0,002-0,15	0,75-54,66	0,62-45,19
		barrage 5 m	102	12-11,76	42-41,17	48-47,06	6,54	0,09-1,38	1,85-28,29	4,6-70,34
		barrage 10 m	38	10-25,64	19-48,72	10-25,64	0,19	0,007-3,74	0,08-42,78	0,1-53,47
2	été	entrée	6	4-68,0	1-16,0	1-16,0	0,0356	0,0054-15,0	0,0288-81,0	0,0015-4,0
		barrage 0 m	21	1-5,0	9-43,0	11-52,0	0,3479	0,0001-0,0	0,212-57,0	0,163-43,0
		barrage 5 m	127	2-2,0	34-26,0	92-72,0	2,2961	0	0,9911-43,0	1,305-57,0
		barrage 10 m	37	3-8,0	9-24,0	25-68,0	0,5412	0,0108-2,0	0,166-31,0	0,370-68,0
3	automne	entrée	16	1-6,0	9-56,0	6-38,0	0,152	0,0009-0,0	0,098-64,0	0,054-36,0
		barrage 0 m	26	1-4,0	19-70,0	7-26,0	1,07	0,0005-0,0	0,91-85,0	0,16-15,0
		barrage 5 m	48	10-21,0	26-55,0	11-24,0	1,527	0,007-1,0	1,42-93,0	0,1-6,0
		barrage 10 m	28	0	0	28-100,0	0,3	0	0	0,3-100,0

Parmi les espèces fréquemment existants on mentionne:
Testacea : Difflugia tuberculata;
Ciliata : Strombilidium minimum, Vorticella microstoma;

Rotatoria: Brachionus quadridentatus brevipes, Euchlanis dilatata,
Habrothroca constricta, Keratella valga, Polyarthra remata,
Trichocerca similis;
Copepoda:Mesocyclops crassus: nauplii, copépodes st.I et II;
Cladocera: Alona rectangulata, Bosmina longirostris, Daphnia cucculata,
Diaphanosoma orghidani, Leydigia leydigi.

Les densités numériques des zooplanctons ont varié chaque saison à l'entrée dans le barrage et selon les profiles de profondeur.

Par conséquent, le zooplancton a été mieux développé au barrage qu'à l'entrée dans le lac, en confirmant l'évolution favorable du plancton dans les écosystèmes lentiques comparatif à ceux lotiques.

4. CONCLUSIONS

Les modifications majeurs générées sur les biotopes aquatiques par la pollution, les constructions hydrotechniques, etc. ont conduit à la restructuration des biocénoses aquatiques, à la diminution de la biodiversité et souvent à une grande augmentation de la biomasse des algues.

Les principales communautés d'organismes (phytoplancton, zooplancton) du lac d'accumulation de Budeasa ont été mieux développées dans la zone de stabilité du biotope (barrage) que dans la zone d'entrée, en confirmant le rôle principal du plancton concernant la caractérisation de l'eau du point de vue biologique dans les nouvelles conditions crées.

L'accroissement de l'apport anthropogène de substances nutritives à cause de la pollution de l'air, de la fertilisation du sol, du déchargement des eaux usées ménagères et industrielles, a comme résultat l'augmentation de la masse du phytoplancton et de la végétation aquatique, déterminant l'intensification du procès d'eutrophication ayant des conséquences négatives sévères sur la qualité de l'eau et donc sur les possibilités de l'utiliser.

Compte-tenant des valeurs de l'indicateur – biomasse phytoplanctonique – on peut apprécier qu'en 1997, le lac Budeasa a eu une évolution oligo-mésotrophe ayant des tendances vers l'eutrophication.

À la suite des recherches amples effectuées par G. Ivancea (1975), A. Lungu (1990-1998), D. Georgescu, I. Fazekaş, M. Florescu, on peut affirmer que le lac Budeasa, tout comme les autres accumulations construites en cascade sur la rivière Argeş, même si l'apport de nutriments est grand, les manifestations d'eutrophication

sont rares grâce au temps réduit de rétention de l'eau; ce fait pourra être considéré positif si on prend en considération la fonction principale du lac Budeasa, c'est-à dire l'alimentation en eau potable de la ville Piteşti.

BIBLIOGRAPHIE

1. Botnariuc N.. Vădineanu A., 1982, *Écologie*, Les Éditions Didactique et Pédagogique de Bucarest.
2. Georgescu M. et collaborateurs, 1978, Schéma d'aménagement du cours moyen de la rivière Argeş. Lac d'accumulation de Budeasa, Hydrotechnique, 23.
3. Lungu A., Zglobiu M., 1996, Évolution des facteurs physico-chimiques et biologiques dans certaines accumulations sur la rivière Argeş, comme résultat des aménagements hydrotechniques. Études et recherches scientifiques, Université de Bacău II: 189-196.
4. Lungu A., 1999, *Thèse de doctorat, Influence des aménagements hydrotechniques sur les cénoses planctoniques sur la rivière Argeş*, Université Ovidius de Constanţa.
5. Vădineanu A.. 1998. *Développement durable*, Les Éditions de L'Université de Bucarest.

Reçu le 20 août, 1999.

L'Institut National de Recherches-Développement pour la Protection de L'environnement. Splaiul Independenţei, 294. Bucarest

LA DISTRIBUTION ALTITUDINALE DES ESPÈCES DE THYSANOPTÈRES (INSECTA: THYSANOPTERA) DU MASSIF GÂRBOVA

LILIANA VASILIU-OROMULU

The work presents the vertical distribution of thysanoptera species from the secondary meadows in the Gârbova Massif, differentiated in altitude in the medium and upper mountain levels. We have done a comparative study of the altitude distribution of praticolous thysanoptera from the Gârbova and Bucegi Massifs.

1. INTRODUCTION

Entre les divers types d'écosystèmes naturels que les thysanoptères peuplent, les prairies montagneuses des zones tempérées présentent la diversité la plus riche d'espèces de thrips.

Dans les Alpes autrichiens, à des altitudes différentes on a signalé 35 espèces (Pelikán, 1996), en Suède 23 espèces (Oettingen, 1954), en Allemagne 37 espèces (Oettingen, 1942) et en Roumanie, dans le Massif Bucegi, 27 espèces (Knechtel, 1963).

2. MATÉRIEL ET MÉTHODE

Dans le Massif Gârbova, dans la Vallée de Bogdan et dans le mont Setu on a établi six stations de prairie secondaire, différenciées par altitude: l'étage de montagne moyen (entre 800–1400 m), l'étage de montagne supérieur (1500 m), et plateau de montagne (Fig. n° 1).

Les prairies secondaires ont eu un régime de réserve naturelle parce qu'on ne les a pas fauchées et broutées au long des années. Les stations situées à 800 m. 900 m. 1050 m et 1200 m sont caractérisées par l'association ***Festuco rubrae-Agrostetum capillaris*** Horv. 51, la station située à 1400 m par l'association ***Hieracio rotundati-Piceetum abietis*** Pawl. Et Br. 39, et celle de 1500 m appartient à l'association ***Violo declinatae-Nardetum*** Simon 66.

Dans les recherches écologiques en stationnaire, faites pendant 3 années consécutives et ultérieurement, en régime de monitoring, les thysanoptères ont été collectées en fauchant avec le filet entomologique et en secouant les plantes en fleur, méthodes consacrées mondialement.

3. RÉSULTATS ET DISCUSSIONS

Dans le Massif Gârbova on a collecté 78 espèces, donc un pourcentage de 36,28% du total des espèces de la faune de la Roumanie (Vasiliu-Oromulu, 1998).

REV. ROUM. BIOL.–BIOL. ANIM., TOME 44, N° 2, P. 131–138, BUCAREST, 1999

D'entre eux 8 espèces appartiennent à la famille Aeolothripidae, 51 espèces à la famille Thripidae et 19 espèces à la famille Phlaeothripidae (tableau n° 1). Du point de vue trophodynamique les thrips appartiennent à deux modules, et précisément, 69 espèces sont consommateurs primaires, et 9 espèces consommateurs secondaires.

On remarque, dans le tableau n° 1, que, pour les espèces *Rhipidothrips gratiosus*, *Bolothrips bicolor* et *Haplothrips phyllophylus*, l'altitude de 800 m constitue la limite supérieure de la répartition verticale dans le Massif Gârbova; pour les espèces *Phlaeothrips pillichianus* et *Chirothrips aculeatus* la limite est de 900 m; *Melanthrips knechteli*, *Frankliniella tenuicornis* et *Sminyothrips biuncatus* apparaissent seulement à la hauteur de 1200 m, *Kakothrips dentatus* à 1400 m, tandis que la relique tertiaire, *Apterothrips secticornis*, à répartition boréale-montagneuse (Vasiliu-Oromulu, 1998) à la limite inférieure de sa répartion, à l'altitude de 1500 m. La majorité des espèces présente une ample distribution verticale.

Les recherches écologiques en stationnaire, faites par K.W.Knechtel (1956, 1963) dans le Massif Bucegi, dans deux prairies secondaires situées à 1163 m et 1500 m (Piatra Arsa), ont permis la comparaison entre la distribution verticale des espèces de thrips avec d'autres, d'altitude similaire du Massif Gârbova et caractérisées par la même association végétale.

Ainsi, il y a des espèces de thrips seulement dans les stations des prairies du Massif Gârbova caractérisées par différences d'altitude: *Aeolothrips albicinctus* (800-1400 m), *Melanthrips knechteli* (1200 m), *Firmothrips firmus* (900–1400 m). *Kakothrips dentatus* (1400 m), *Limothrips schmutzi* (900–1500 m). *Neohydatothrips abnormis* (900–1200 m), *Odontothrips confusus* (1050–1400 m). Ici on a signalé des espèces de thrips identifiées dans le Massif Bucegi à une répartition verticale étroite: *Chirothrips aculeatus* (900 m), *Haplothrips kurdjumovi* (800–900 m), *Hoplandrothrips bidens* (900–1050 m) et *Phlaeothrips pillichianus* (900 m) (Knechtel, 1956, 1963).

Leur présence dans la structure des associations de thysanoptères praticole est accidentale, elles étant amenées par le vent de la zone forestière.

D'autres espèces arboricoles, identifiées dans le Massif Bucegi. dans les écosystèmes forestiers, aparaissent dans le Massif Gârbova, dans les écosystèmes de prairies, répandues anémochoriquement mais à altitudes différentes.

Ainsi, *Oxythrips bicolor*, apparaît ici entre 1200–1400 m et dans le Massif Bucegi à 900 m et *Phlaeothrips coriaceus* à 1100 m respectif à 900 m (tableau n° 2).

Il y a dans le Massif Gârbova des espèces de thrips qui se trouvent à altitudes beaucoup plus grandes dans le Massif Bucegi. Ainsi, l'espèce graminicole *Frankliniella tenuicornis*, limitée dans le Massif Gârbova à 1200 m. dans le Massif Bucegi a été signalée jusqu'à 2500 m. *Thrips pillichi*, dans le Massif Gârbova atteint seulement 1050 m, dans le Massif Bucegi monte jusqu'à 1800 m.

Tableau 1 (à suivre)

La distribution altitudinale des espèces de Thysanoptères du Massif Gârbova

altitude	800 m	900 m	1050 m	1200 m	1400 m	1500 m
1	2	3	4	5	6	7
Fam. Aeolothripidae						
Aeolothrips albicinctus	+	+	+		+	
Aeolothrips ericae	+	+	+	+	+	+
Aeolothrips fasciatus	+	+	+	+	+	+
Aeolothrips intermedius	+	+	+	+	+	+
Melanthrips fuscus	+	+	+	+	+	+
Melanthrips knechteli				+		
Melanthrips pallidior	+	+	+	+	+	+
Rhipidothrips gratiosus	+					
nombre des espèces	7	6	6	6	6	5
Fam. Thripidae						
Anaphothrips euphorbiae		+				+
Anaphothrips obscurus	+		+	+	+	+
Apterothrips secticornis						+
Aptinothrips elegans	+	+	+	+	+	+
Aptinothrips rufus	+	+	+	+	+	+
Aptinothrips stylifer	+	+	+	+	+	+
Chirothrips aculeatus		+			+	
Chirothrips manicatus	+	+	+	+	+	+
Firmothrips firmus	+	+	+	+	+	
Frankliniella intonsa	+	+	+	+	+	+
Frankliniella pallida	+				+	
Frankliniella tenuicornis				+		
Kakothrips dentatus					+	
Kakothrips robustus	+	+	+	+	+	
Limothrips denticornis	+	+	+	+	+	+
Limothrips schmutzi	+	+		+		+
Mycterothrips annulicornis	+		+			
Neohydatothrips abnormis	+	+		+		
Odontothrips biuncus	+	+	+	+		+
Odontothrips confusus			+	+	+	
Odontothrips loti	+	+	+	+	+	+
Odontothrips phaleratus		+	+	+		
Oxythrips bicolor				+	+	
Parafrankliniella verbasci				+	+	
Prosopothrips vejdovski	+			+		+
Sericothrips bicornis	+	+	+			
Sminyothrips biuncatus				+		
Stenothrips graminum						+
Taeniothrips inconsequens		+			+	

Tableau 1

1	2	3	4	5	6	7
Taeniothrips picipes	+	+	+	+	+	+
Tenothrips discolor					+	
Tenothrips frici	+	+	+	+	+	+
Thrips atratus	+	+	+	+	+	+
Thrips crassicornis				+		
Thrips dilatatus						+
Thrips euphorbiae				+	+	+
Thrips flavus	+	+	+	+	+	
Thrips incognitus					+	
Thrips major	+	+		+		+
Thrips minutissimus		+	+	+	+	+
Thrips montanus	+	+	+	+	+	+
Thrips montivagus	+	+	+	+	+	
Thrips nigropilosus	+	+		+		
Thrips pelikani	+	+	+	+	+	+
Thrips physapus	+	+	+	+	+	+
Thrips pillichi	+		+			
Thrips tabaci	+	+	+	+	+	+
Thrips trehernei	+	+	+	+	+	+
Thrips trybomi	+	+	+	+	+	
Thrips validus	+	+	+	+	+	+
Thrips vulgatissimus	+	+	+	+	+	+
nombre des espèces	33	33	32	39	34	25
Fam. Phlaeothripidae						
Bolothrips bicolor	+					
Haplothrips acanthoscelis	+	+	+	+	+	+
Haplothrips aculeatus	+	+	+	+	+	+
Haplothrips alpester	+	+	+	+	+	+
Haplothrips angusticornis	+	+	+	+	+	+
Haplothrips distinguendus	+	+	+	+	+	+
Haplothrips kurdjumovi	+	+				
Haplothrips leucanthemi	+	+	+	+	+	+
Haplothrips niger	+	+	+	+	+	+
Haplothrips phyllophilus	+					
Haplothrips reuteri	+	+	+	+	+	+
Haplothrips setiger	+	+	+		+	+
Haplothrips subtilissimus	+	+		+	+	
Haplothrips tritici	+	+	+	+	+	+
Hoplandrothrips bidens		+	+			+
Liothrips austriacus				+	+	
Liothrips setinodis		+		+		+
Phlaeothrips coriaceus		+				
Phlaeothrips pillichianus		+				
nombre des espèces	4	6	11	2	2	4

Tableau 2

Les espèces de thysanoptères présentes dans les prairies altitudinales du Massif Bucegi

1200 m (Knechtel, 1956)	1500 m (Knechtel, 1963)
Fam. Aeolothripidae	**Fam. Aeolothripidae**
Aeolothrips intermedius	*Aeolothrips fasciatus*
Aeolothrips ericae	*Aeolothrips intermedius*
Aeolothrips verbasci	*Melanthrips fuscus*
Melanthrips fuscus	
Melanthrips pallidior	**Fam. Thripidae**
Fam. Thripidae	*Aptinothrips rufus*
Chirothrips manicatus	*Aptinothrips stylifer*
Odontothrips biuncus	*Chirothrips manicatus*
Oxythrips ulmifoliorum	*Odontothrips loti*
Platythrips tunicatus	*Oxythrips ulmifoliorum*
Rubiothrips silvarum	*Taenithrips picipes*
Taenithrips picipes	*Tenothrips discolor*
Thrips atratus	*Thrips incognitus*
Thrips euphorbiae	*Thrips atratus*
Thrips montanus	*Thrips flavus*
Thrips montivagus	*Thrips minutissimus*
Thrips physapus	*Thrips montanus*
Thrips tabaci	*Thrips nigropilosus*
Thrips trybomi	*Thrips physapus*
Thrips vulgatissimus	*Thrips tabaci*
	Thrips trehernei
Fam. Phlaeothripidae	*Thrips validus*
Haplothrips angusticornis	*Thrips vulgatissimus*
Haplothrips leucanthemi	**Fam. Phlaeothripidae**
Haplothrips niger	*Haplothrips acanthoscelis*
Haplothrips nigricans	*Haplothrips alpester*
	Haplothrips angusticornis
	Haplothrips leucanthemi
	Haplothrips niger
	Haplothrips reuteri

À l'altitude de 1163 m dans le Massif Bucegi, on a identifié 23 espèces (Knechtel, 1956), tandis que dans le Massif Gârbova, à 1200 m. 56 espèces. seulement 26 espèces sont communes aux deux stations.

À 1500 m, dans le Massif Bucegi, le tableau spécifique contient 23 espèces communes, des 27 signalées ensommant les thrips du Massif Gârbova. qui ont une diversité plus grande: 47 espèces.

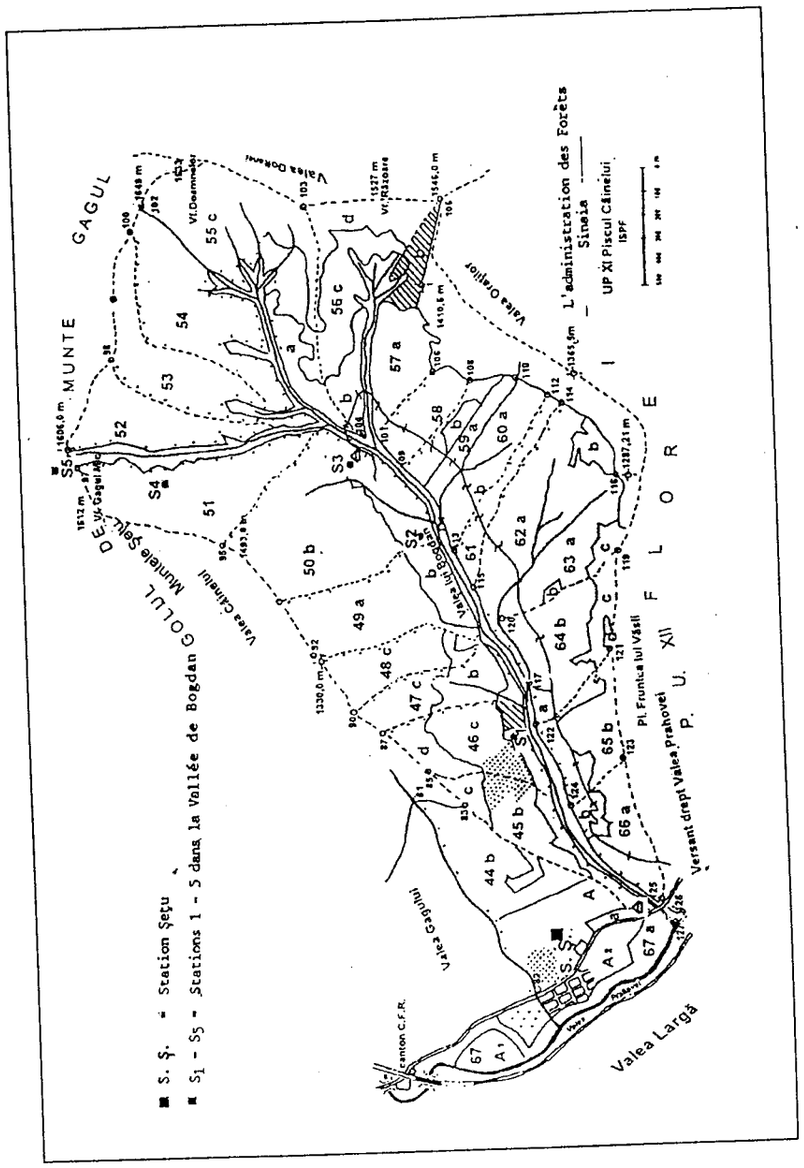


Fig. 1 – La carte des stations du Massif Gârbova.

On remarque la richesse des espèces de thrips des praires différenciées selon l'altitude du Massif Gârbova, que l'on doit à la structure des associations végétales qui constituent le support trophique de ces consommateurs, aux facteurs mécaniques: l'intensité réduite du vent, les précipitations annuelles moyennes réduites, de 800–1100 mm, l'indice d'aridité de Martone est supérieur au 40, en comparaison avec le Massif Bucegi où les précipitations atteignent 1000–1500 mm, l'indice d'aridité de Martone dépasse 50.

La structure des associations de thrips dans chaque station est due à la compétition, résultée d'un mélange d'habiletés d'exploiter l'environnement, ainsi que la présence de certaines espèces de thysanoptères, qui peut être considérée un facteur important pour établir les principaux mécanismes impliqués dans le maintien de la stabilité des écosystèmes.

4. CONCLUSIONS

La distribution verticale des thysanoptères du Massif Gârbova indique une limite d'altitude dans la répartition de certaines espèces. Ainsi, pour la répartition verticale des espèces *Rhipidothrips gratiosus*, *Bolothrips bicolor* et *Haplothrips phyllophylus* l'altitude de 800 m constitue la limite supérieure, et pour la relique tertiaire. *Apterothrips secticornis*, l'altitude de 1500 m constitue la limite inférieure.

Faisant une comparaison entre les prairies secondaires de la même altitude et caractérisées par la même association végétale du Massif Bucegi (Knechtel, 1956, 1963) la limite verticale de la répartition de certaines espèces de thysanoptères du Massif Gârbova est plus élevée.

À la même altitude la diversité spécifique à été beaucoup plus grande dans le Massif Gârbova, probablement à cause des températures plus élevées, des précipitations et de l'intensité du vent, plus réduites, en comparaison avec le Massif Bucegi.

BIBLIOGRAPHIE

1. Knechtel, W. K., 1956. *Ökologisch-phaenologische Forschungen über Thysanopteren,* X Intern. Kongress of Ent. Montreal, II. 689–695.
2. Knechtel, W. K., 1963. *Ökologisch-phaenologische Forschungen über Thysanopteren. Zur Kenntnis der Thysanopterenfauna der Karpathen.* Beiträge zur Ent., **13,** *3/4*, 369–377.
3. Oettingen, H., VON, 1942. *Die Thysanopteren des norddeutschen Grasslands.* Ent. Beih. Berl. Dahlem. **9**. 79–141.
4. Oettingen, H., Von, 1954. *Beiträge zur Thysanopterenfauna Schwedens,* Ent. Tidskr., **75**, (*2–4*), 134–150.

5. Pelikán, J., 1996, *Vertical distribution of alpine Thysanoptera,* Folia Ent. Hung., LVII, 121–125.
6. Vasiliu-Oromulu, Liliana, 1993, *Changes in the structure and dynamics of the Thysanoptera populations in the pasture ecosystems of subalpine areas of Bucegi Mountains subjected to the ecological reconstruction*, in "Advances in Thysanopterology", Sc. Publ. New Delhi. 1–8.
7. Vasiliu-Oromulu, Liliana, 1998, *Lista revizuită a speciilor de thysanoptere din România.* St. Cerc. Biol. s. Zool., **2**, 77–83.
8. Vasiliu-Oromulu, Liliana, 1998, *Aspecte ecologice ale thysanopterelor din Romania*, St. Cerc. Biol. s. Zool., **2**, 85–96.
9. Vasiliu-Oromulu, Liliana, 1999, *Endemits, relicts and vicariant Thysanoptera species from Romania (Insecta: Thysanoptera)*, Entomol. Rom., **3**, 59-62, Cluj.

Reçu le 1er Septembre, 1999.

Institut de Biologie
Splaiul Independenţei 296
Bucarest

DESIGNING AND USING OF A DATA ACQUISITION SYSTEM FOR PARALLEL MONITORING OF BOTH BIOELECTRICAL AND MECHANICAL ACTIVITIES OF ISOLATED FROG HEART

C. SEVCENCU*, A. PATRICIU**, C. ARDELEAN*

In order to record from single isolated frog heart two different parameters at the same time, a computer-based data acquisition system (DAQS) was designed. A data acquisition board receives both the electrical signals generated by the ventricle, and the coordinates of its mechanical cycle. These two value strings are then transmitted to the PC by means of a proper software package, which also ensures the subsequent analyzing process. Taking into consideration that our recordings are comparable to those currently obtained in other laboratories and reflect well enough any change of the cardiac activity, we consider our DAQS as a valuable tool for an integrated study of both the electrical and the mechanical cycles of the frog heart.

1. INTRODUCTION

The first step towards the study of certain electrical signals generated by the heart became possible in the 1880s, after the construction of a device (i.e. the Lippmann capillary electrometer) capable to acquire voltage fluctuations from living structures. To record the traces for further analysis, photographic methods were used [26].

The transposing of the traces reflecting a bioelectric process on film or photographic paper directly from the screen of the oscilloscope [12] has been for many years the single recording method and it is still used [1, 17]. The construction of memory oscilloscopes made possible the storage of the traces and their reactivation for subsequent measurements [6, 7, 11].

During the last decade, a new generation of computer-based data acquisition methods has been developed and they are widely used today in the field of cardiac physiology, either related to mechanical parameters or to electrical ones.

These methods are probable the most suitable ones regarding mainly the electrophysiological research tasks.

Using an optical method to record electrical signals from the surface of the frog ventricle, Neunlist *et al.* [15] monitored integrated action potentials by means of a LabVIEW-2 software package. As they mention, although it is a non-invasive method, the use of potentiometric dyes to record the transmembrane potential has some clear disadvantages, such as the presence of the motion artifacts, limited signal-to-noise ratio, lack of an absolute voltage reference, possible toxicity by the dye and/or its solvent, and photodynamic damage to the tissue.

REV. ROUM. BIOL. – BIOL. ANIM., TOME 44, N° 2, P. 139–149, BUCAREST, 1999

As we have previously reported [18–20], an original DAQS for monitoring electrical signals generated by the frog ventricle was designed and built in our laboratory. In comparison with the above mentioned technique, using a steel electrode and a proper tensioning device, the motion artifacts were totally eliminated, the noise level was also reduced and no toxicity problems affected the ventricular tissue.

Both related to single cells and to multicellular preparations, the experiments are mainly performed either to monitor electrical signals [4, 5, 15, 25] or contractile processes [9, 14, 21-24].

Taking into consideration that the mechanical activity of the myocardium is still a problem of interest in the field of general cardiac physiology [9, 16, 21-24], and that it is closely related to the electrical one, we have tried to simultaneously monitor these both phenomena.

By adding a self-made displacement transducer into the perfusion device of the previously mentioned data acquisition system, we have obtained our actual double trace DAQS, which will be further presented.

2. MATERIALS AND METHODS

Animals. The size of the frogs is not important because the fitting devices from the perfusion chamber are adaptable to the heart length.

The data acquisition system. Our system consists of five main modules. These are: the perfusion module – which ensures the proper conditions for the heart activity, the signal amplifier – which increases the signal amplitude to the requested value, the displacement transducer – which acquires the ventricular movements, the data acquisition (DAQ) board – which pre-processes and transmits the data to an IBM compatible PC – the fifth component of the DAQS (see, for illustration, Fig.1).

The perfusion module consists of a perfusion tank -1) and a perfusion device. Each reservoir (R1,R2 and R3) of the perfusion tank can contain any requested solution, one of them being always the Ringer one for the reference cardiac activity. The Ringer solution can be instantly replaced by one from the other reservoirs by manipulating their taps -2).

Three plastic tubes -3) connect the reservoirs at the perfusing head -4), where the cardiac cannula -6) will also be fitted. Made from steel, this cannula also plays the role of the reference electrode and it is connected at the signal amplifier by means of an electric cable. A displacement device -5) adapts the position of the displacement transducer to the heart size, thus ensuring the proper tensioning of the heart.

The active electrode -7) consists of a hook which can be fitted to the mobile device of the displacement transducer by means of a connector which also realizes the link between the electrode and the signal amplifier.

The hook end of this electrode stabs the tip of the ventricle and acquires the electrical activity of the tissue. The elastic force within the transducer ensures a counter-force, which opposes to the ventricular contraction and brings the heart to its initial length during the relaxing period.

The amplifier of a Cardior KTD2 apparatus was used for signal amplification and its exit was linked to one entrance of the DAQ board.

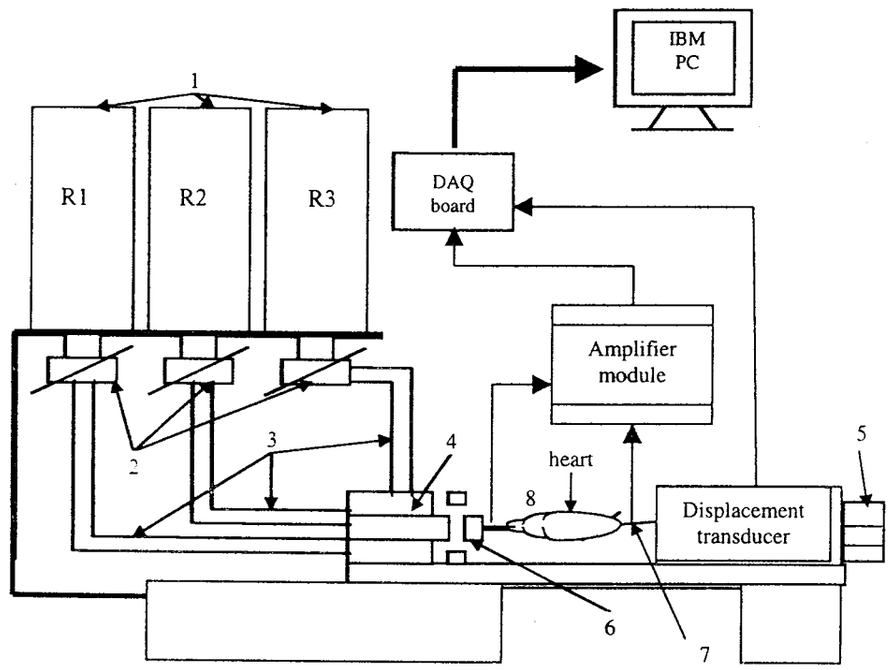


Fig.1 – Schematic diagram of the data acquisition system.1) – Perfusing reservoirs. 2) – Taps. 3) – Plastic tubes. 4) – Perfusing head. 5) – Displacement device. 6) – Perfusion cannula/reference electrode. 7) – Active electrode. 8) – Perfusion chamber.

The displacement transducer translates the ventricular movements during the mechanical cycle into electrical parameters, which are further also transmitted to the DAQ board.

Thus, both the electrical signals generated by the ventricle and its translated movements reach the computer via DAQ board.

The DAQ board is an electronic device which plays three crucial roles in the acquisition process: the measurement of the signals acquired from the transducer, those of a digital voltmeter and those of a transmission element.

The heart preparation begins with the isolation procedures, which were previously presented [19].

Related to the new design of the actual perfusion chamber, after fitting the cannula at the perfusion head, the connector of the hook-electrode must also be fixed into its proper place on the displacement transducer.

By manipulating its displacement element, the device containing the displacement transducer is positioned according to the length of the heart. Thus, at the end of these operations, the organ must stay in horizontal position, gently tensioned between the cardiac cannula and the active electrode (see Fig.1).

The two electrodes are then connected to the cables of the amplifier module. Starting from this moment, the self-pacing bioelectrical activity of the ventricular tissue may be watched on the screen of the ECG.

After the stabilization of heart beating, the PC monitoring may start by triggering the acquisition program.

3. RESULTS AND DISCUSSIONS

The perfusion device. In chronological order, the first result of our work was the realisation of an original perfusion device and its main performances were already presented [19].

Related to the actual acquiring tasks, only few changes were performed.

The most important is the adding of the displacement transducer, which allows the acquisition of the ventricular movements parallel to its electrical activity.

Another new element of our system is the mobility of the device containing the displacement transducer. Taking into consideration that the hearts may have different sizes, we had to think of a technical formula in order to ensure the adaptability of the attachment devices to the heart length. Thus, instead of fixing the transducer into the perfusion chamber, we have connected it to a thread based displacement device, which ensures its foreword and backward movements, as the heart length would require.

When one has to record signals generated by a moving cell, tissue or organ, a major problem is the attachment of the electrodes so that the motion artefacts should be reduced or eliminated. Different methods have been established to solve this demand and we mention here the using of suction [13-15] or the calcium removal in order to inhibit the contractions [13].

Part of our solution to this problem is based on using a metallic cannula instead of the classical glass one. Thinking that anyhow the cannula must be strongly bound to the sinus wall, we decided to use it as a reference electrode.

The other half of the problem, that of a proper and stable contact between the ventricular tissue and the active electrode, was solved by means of a proper design of both the active electrode and the transducer.

After the isolation, the heart with the electrode hanging on its tip is attached to the perfusing head by means of the cardiac cannula. The second attachment point is represented by the connector of the electrode, by means of which this will be fitted at the mobile element of the displacement transducer (see the description above and Fig. 1). The next operation is to move backward the device containing the transducer till its counter-force spring remains gentle tensioned even during the relaxing period. This state of permanent tension ensures the indispensable tissue-electrode contact along the whole cardiac cycle. The elastic force accumulated by the spring during the contraction also serves for bringing the heart back to its initial length.

The DAQ board. We have mentioned before that this electronic device was designed to ensure both the digitisations of the signals and their transmission to the PC.

As a digital voltmeter, the DAQ board receives the amplified signals and converts them into numerical values.

As a frequency-meter, the DAQ board measures the frequency of the oscillator, which is the heart of the displacement transducer, and also transforms it into numerical values.

As a transmission element, the DAQ board sends in real time the converted data to the PC by means of its serial interface.

The software package. The data acquisition and processing are performed by means of a self-designed, LabView-based software package which consists of two parts.

One of the programs receives the value strings sent by the serial interface and transforms them into analog graphic representations. Thus, the two traces rule on the monitor almost in real time. Parallel to this processing task, the program also saves the data into dedicated files on the harddisk.

The software package also contains a program designed to display the recordings made by the first one. Thus, a wave shape continuous changing process determined, for example, by changing the Ringer solution with one containing a disturbing factor, may be seen from the beginning till the end. Due to the facilities of this program, any segment from such a recording may be zoomed and analyzed.

Recorded signals. To illustrate the performances of our DAQS, we shall further present some of the recordings obtained by using it.

As we have mentioned above, from a whole recording, one could choose and magnify only a small segment.

In Fig. 2, three steps of magnification – a, b and c – can be seen. The c cell shows a single bioelectrical wave recorded from the ventricular myocardium parallel to the ventricular mechanical cycle. According to Weidmann [26], this wave would represent the development of a so-called "injury action potential".

Neunlist *et al.* [15], using an original optical method, obtained an integrated transmembrane potential recorded from frog ventricular epicardium.

Although this signal, being acquired from a volume of tissue approximately 200 μm in diameter, may differ from that of individual cells [13], it expresses the main characteristics of a well-known cardiac action potential. The maximal amplitude was reported to be of about 100 to 120 mV and the total duration of this integrated action potential ranged from 700 to 900 ms.

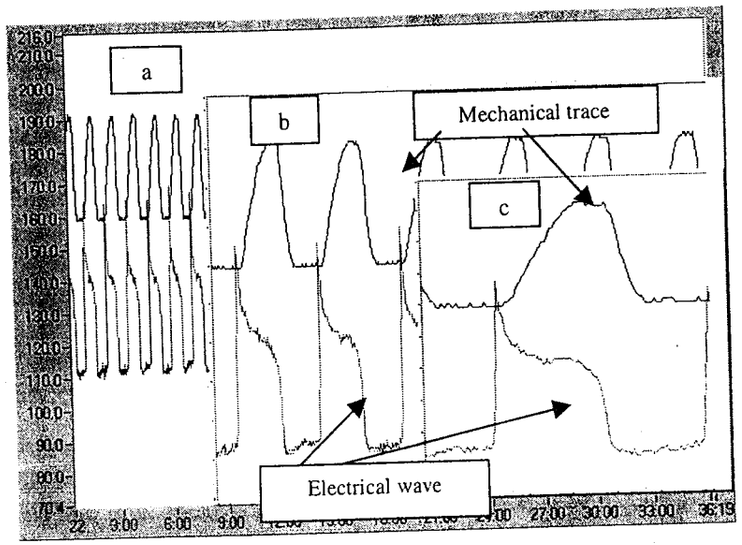


Fig. 2 – Three magnification steps of a double-trace recording acquired from the frog ventricular myocardium.

The individual electrical signals acquired in our laboratory also seem to present the characteristic steps of an action potential and these were described in a previous paper [19].

The signal starts with a rapid up-stroke that was found to last for 25 – 30 ms. The maximal spike amplitude ranges from 25 to 30 mV. After a rapid decrease of the amplitude, the subsequent characteristic plateau follows. Finally, the potential returns to its base-line value by means of a prolonged decreasing period. .

Taking into consideration that the signal was recorded from a cellular mass and according to Delvin [3], who reported spike amplitudes of about only 5 to 9 mV acquired from the ventricle of *Mercenaria mercenaria* also by means of an extracellular recording technique, we explain the differences between the amplitude of our spikes and those measured by Neunlist *et al.* [15] to be due to the short-circuitry processes appeared along the tissue-electrode contact area.

Related to the shape of our electrical signals, we have established three different and well-defined groups of waves acquired from the ventricular myocardium (see Fig. 3).

This finding seems to be consistent with the observations that both ventricular and atrial action potentials express a significant shape heterogeneity. Thus, Courtemanche *et al.* [2] mention three different morphological wave types recorded from isolated human atrial tissue: type 1 – a rectangular wave with a positive plateau, type 2 – a "spike-and-dome" wave and type 3 – a triangular wave with a little plateau. The same shapes were reported by Li *et al.* [8] according to a ventricular transmural wave heterogeneity and, as can be seen in Fig. 3, our wave-types are identical to those mentioned above.

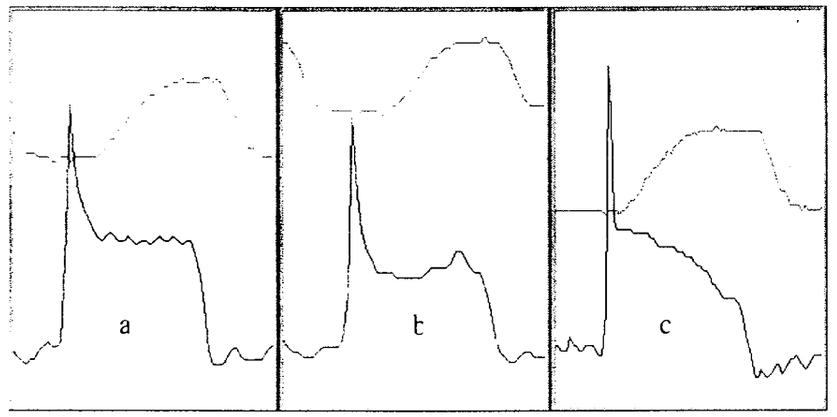


Fig. 3 – Three different wave shapes acquired from the frog ventricular myocardium. a) – rectangular wave; b) – "spike-and-dome" wave; c) – triangular wave.

These three shapes are also characterized by different time parameters.

Thus, three different APD_{50} (action potential duration at 50% repolarization [8]) were measured: 139.2 ms for a) form, 71.7 ms for b) form and 369.2 ms for c) form.

APD_{90} – action potential duration at 90% repolarization, as expected, does not depend on the wave shape, and was established at about 541.3 ms.

Related to the total duration of the waves, our values are in accordance with the limits established by Neunlist *et al.* [15] (see above).

V_{max} – defined as the maximum rate of rise of the action potential [10] – was found to be 1.02 V/s.

As a characteristic of their recording devices, Neunlist *et al.* [15] reported a peak-to-peak noise level of 2 – 7% from the action potential amplitude.

In our recordings, the maximum level of the peak-to-peak noise was 5% of the signal amplitude.

As we have already mentioned, a difficult recording problem is the fixing of the electrodes into/on the tissue when the bioelectrical signals are acquired from a moving organ.

The authors cited above have recorded their signals by means of an optical fiber which was positioned on the surface of the ventricle. When this fiber was not properly fixed by suction, a major motion artifact appeared some 50 - 100 ms after the upstroke of the action potential.

Although our signal is always followed by the ventricular contraction, no motion artifact appears (see Fig. 2). Since the motion artifacts were totally eliminated, we may conclude that our technical solution related to the attachment of the electrodes is a valuable one.

The spike of the electrical signal is followed by the development of the mechanical cycle after an almost constant interval of 90 ms.

For characterizing the mechanical cycles, the classical parameters of a contraction process were determined on Ringer solution perfusion. These are the contraction period – 281 ms, the contraction plateau – 157 ms and the relaxing period – 160 ms.

Starting from the technical characteristics of our displacement transducer and making the requested calibrations, we have also established some new parameters in order to better define the mechanical properties of the ventricular myocardium during the contraction period:

1. the contraction velocity – $v_c = A_c / t_c$; where A_c is the maximal contraction amplitude and t_c is the contraction period: it has a mean value of 5.7 mm/s,
2. the contraction force – $F_c = k A_c$; where k is the elasticity constant of the spring within the displacement transducer; it was found to have a mean value of 1.8 mN,
3. the ventricular work – $L_c = F_c A_c$; with a mean value of 2.85 μJ.
4. the ventricular mechanical power – $P = L_c/t_c$; with a mean value of 10.5 μW.

In comparison to our previous form of a cardiac DAQS, which monitored only the electrical signals generated by the self-pacing ventricle, by means of the present one we are able to record both the potential changes, and the related ventricular contraction. Beside a simultaneous viewing, the facilities of the processing software allow their correlate analysis. Since the ionic movements during the electrical process induce the development of the mechanical one. an integrated study of these phenomena may offer more information about the links between them.

Finally, we illustrate the capacities of our DAQS to reflect the effect of a disturbing factor on both ventricular mechanical and electrical activities.

As can be seen in Fig. 4, a change in the signal and contraction shapes. as well as in frequency, is developing after the application of a substance which affects the cardiac activity. Thus, the picture above shows the transition from a normal self-pacing ventricular activity to that determined by the administration of methanol or Digoxin solved in Ringer solution. One can observe that the temporal

changes of the electrical process underlay similar changes of the contraction periods. Without discussing here the physiological mechanisms of these effects, we only mention that our devices and methods seem to surprise well enough such a development, reflected on both electrical waves and mechanical cycles.

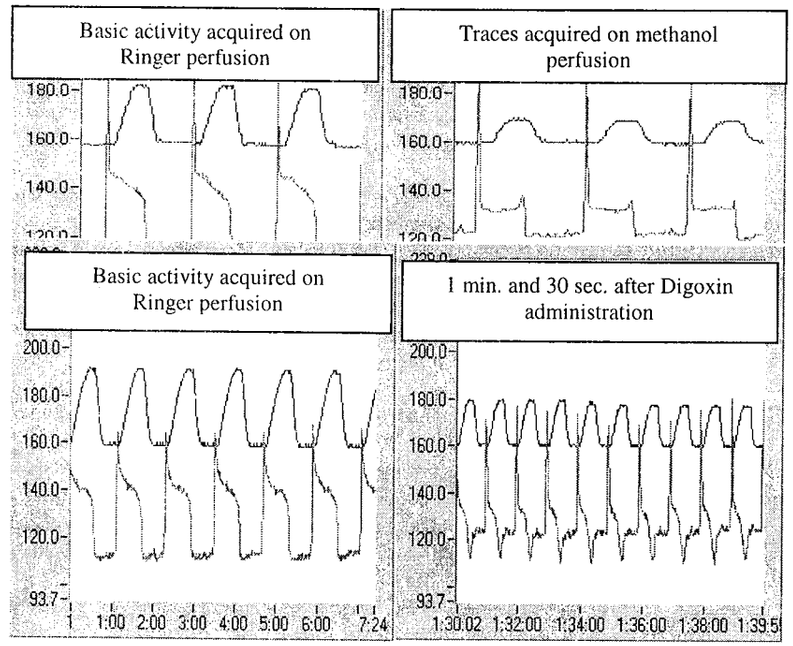


Fig. 4 – Effect of methanol and Digoxin on the mechanical and electrical activity of frog ventricle.

As a **conclusion** of the present work, we believe that our DAQS may be considered a valuable tool for the study of the frog heart bioelectrical activity either alone, or in relation to other cardiac parameters such as the ventricular mechanical cycle.

REFERENCES

1. A. ALLY, S. MURAYAMA, *Cardiovascular effects of central administration of cholinomimetics in anesthetized cats,* Neuropharmacology, **32**, 185–193 (1993).
2. M. COURTEMANCHE, R. J. RAMIREZ, S. NATTEL, *Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model,* Am. J. Physiol. **275** (Heart. Circ. Physiol. *44*). H301-H321 (1998).
3. C. DELVIN, *An analysis of control of the ventricle of the mollusc Mercenaria mercenaria. I. The ionic basis of autorhythmicity,* J. Exp. Biol., **179**, 47–61 (1993).

4. N. FARES, P. BOIS, J. LENFANT, D. POTREAU, *Characterization of a hyperpolarization-activated current in dedifferentiated adult rat ventricular cells in primary culture.* J. Physiol., **506**, 73–82(1998).
5. M. GOETHALS, A. RAESVAN, P. BOGAERT, *Use-dependent block of the pacemaker current I_f in rabbit sinoatrial node cells by zatebradine (UL-FS 49),* Circulation, **88**, 2389–2401 (1993).
6. Y. HARRA, A. ALLY, S. MURAYAMA, *Central cardiovascular effects of physostigmine in anesthetized cats,* Neuropharmacology, **31**, 923–928 (1992).
7. Y. KURACHI, *Voltage-dependent activation of the inward-rectifier potassium channel in the ventricular cell membrane of guinea-pig heart,* J. Physiol., **366**, 365–385 (1985).
8. G.-R. LI, J. FENG, L. YUE, M. CARRIER, *Transmural heterogeneity of action potentials and Ito1 in myocytes isolated from the human right ventricle,* Am. J. Physiol., **275** (Heart Circ. Physiol. *44*), H369-H377 (1998).
9. C.-H. LUO, L. TUNG, *Null-balance transducer for isometric force measurements and length control of single heart cells,* IEEE Trans. Biomed. Eng., **38**, *12*, 1165–1174 (1991).
10. M. C. F. MEIER, G. M. BRIGGS, W. C. CLAYCOMB, *Electrophysiological properties of cultured adult rat ventricular cardiac muscle cells,* Am J. Physiol., **250** (Heart. Circ. Physiol., 5). H731-H735 (1986).
11. T. NAKAJIMA, T. SUGIMOTO, Y. KURACHI, *Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane,* J. Gen. Physiol., **99**. 665–682 (1992).
12. R. NEGOESCU, *Instrumentația electronică biomedicală,* București, Editura Tehnică. 1985. p. 141.
13. M. NEUNLIST, L. TUNG, *Optical recording of ventricular excitability of frog heart by an extracellular stimulating point electrode,* Pace, **17**, 1641–1654 (1994).
14. M. NEUNLIST, L. TUNG, *Dose-dependent reduction of cardiac transmembrane potential by high-intensity electrical shocks,* Am. J. Physiol., **273** (Heart. Circ. Physiol. *42*), H2817-H2825 (1997).
15. M. NEUNLIST, S. ZOU, *Design and use of an "optrode" for optical recordings of cardiac action potentials,* Pflügers Arch., **420**, 611–617 (1992).
18. S. S. PARIKH, S.-Z. ZOU, L. TUNG, *Contraction and relaxation of isolated cardiac myocytes of the frog under varying mechanical loads,* Circ. Res., **72**, 297–311 (1993).
17. D. S. RUBENSTEIN, L. M. FOX, J. A. MCNULTY, S. L. LIPSIUS, *Electrophysiology and ultrastructure of Eustachian ridge from cat right atrium: a comparison with SA node.* J. Mol. Cell Cardiol., **19**, 965–976 (1987).
18. C. SEVCENCU, *Effects of certain cardioinhibiting and cardioactivating factors reflected in the total bioelectrical activity of frog isolated heart,* IV-th National Conference of Biophysics. Cluj-Napoca, October 1997, Abstracts, P-33.
19. C. SEVCENCU, A. PATRICIU, E. CHIHAIA, *System for monitoring the total bioelectrical activity of frog isolated heart,* IV-th National Conference of Biophysics, Cluj-Napoca. October 1997, Abstracts, P-34.
20. C. SEVCENCU, A. PATRICIU, *An original data acquisition system for monitoring the bioelectrical activity of frog heart,* Studia Universitatis Babeș-Bolyai. Biologia. **XLV**. *(1)*. 103–112 (1999).
21. D. A. SYME, *Influence of extend of muscle shortening and heart rate on work from frog heart trabecule,* Am. J. Physiol. **265** (Regulatory Integrative Comp. Physiol. *34*), R310-R319 (1993).
22. D. A. SYME, *The efficiency of frog ventricular muscle,* J. Exp. Biol., **197**, 143–164 (1994).
23. D. A. SYME, R. K. JOSEPHSON, *Influence of muscle length on work from trabecular muscle of frog atrium and ventricle,* J. Exp. Biol., **198**, 2221–2227 (1995).

24. L. TUNG, M. MORAD, *Contractile force of single heart cells compared with muscle strips of frog ventricle,* Am. J. Physiol. **255** (Heart. Circ. Physiol. *24*), H111-H120 (1988).
25. M. VORNANEN, J. TUOMENNORO, *Effects of acute anoxia on heart function in crucian carp: importance of cholinergic and purinergic control.* Am. J. Physiol., 277 (Regulatory Integrative Comp. Physiol. *46*), R465-R475 (1999).
26. S. WEIDMANN, *Cardiac cellular electrophysiology: past and present,* Experentia, **43**, 133–146 (1987).

Received July 19, 1999.

**Babeș-Bolyai University*
Department of Animal Physiology
Clinicilor 5-7
3400 Cluj-Napoca
Romania

***Aalborg University,*
Centre for Sensory-Motor Interaction,
9220 Aalborg,
Denmark

RECHERCHES SUR L'UTILISATION DE L'ACTIVITÉ TRANSAMINASIQUE (GOT) DANS L'ÉVOLUTION DE LA QUALITÉ DU SPERME DE VERRAT PAR RAPPORT AU RÉGIME DE RÉFRIGÉRATION ET DE CONGÉLATION*

AL.G. MARINESCU[1,4], DANA RICHTER[2]
DIANA DINU[3], Ş.ANDREUŢĂ[4], A. MARINESCU[2]

A positive relationship between the injury degree of pig spermatozoa, following the freezing/decongelation processes, and the activity of the GOT enzyme in the seminal plasma was found. The value of the enzyme activity after decongelation was for the fresh sperm lower (177,90 mU/ml plasma) and for the "Hülsenberg method" sperm higher (300.200 mU/ml plasma). For 1-5 days at 5-7^{0}C a low GOT activity pointed out a reduced degree of the membrane injury.

1. INTRODUCTION

Dans la littérature de spécialité on trouve toute une série de travaux sur la modification de l'activité de certaines enzimes normalement présentes dans le sperme, par rapport au degré d'altération des spermatozoïdes (de leur membrane). De telles recherches ont été d'abord réalisées chez le taureau (PACE et GRAHAM, 1970; FLIPSE, 1960, etc.) une corrélation entre l'intensité de l'activité GOT (transaminase et la baisse de la capacité fécondante des spermatozoïdes étant mise en évidence (SAACKE *et al.*, 1968; POLGE, 1971).

Chez le verrat, des déterminations de ce type ont été effectuées par CRABO *et al.* (1970), GRAHAM *et al.* (1971), HILLMANN (1972), HILLMANN et BADER (1973), les résultats ayant indiqué une corrélation positive entre le degré d'altération de l'intégrité de la cellule spermatique et l'activité de cette enzyme.

Dans le présent travail, nous nous proposons d'exposer les résultats obtenus dans des conditions de réfrigération et, respectivement, de congélation du sperme.

2. MATÉRIEL ET MÉTHODE

Pour un nombre de six échantillons de sperme provenant des verrats des races Landrance et Le Grand Blanc du Complexe d'élevage porcin (SCCCP) de Periş, en différentes variantes et phases de réfrigération et de congélation, on a déterminé l'activité de l'enzyme GOT (L-aspartate: 2-oxoglutarate-aminotransphérase, EC 2.6.1.1.) dans

* Pour cette expérience, certains matériaux (équipements et réactifs) provenant d'une donation de la Fondation Alex. Von Humboldt (Allemagne) ont été utilisés.

le plasma séminal selon un procédé propre représentant une modification de la méthode décrite par Bergmeyer et Bernt (1970), après Karmen (1955), pour les valeurs de cette enzyme dans le sérum sanguin (MARINESCU *et al.*, 1977). L'analyse de l'activité GOT a été faite à l'aide du photomètre type Eppendorf, muni d'un mécanisme enregistreur de compensation, à 366 nm, dans des cuves à température constante (ET=25°C).

En tant qu'enzyme indice on a utilisé la malico-déhydrogénase et on a enregistré la baisse de l'extinction en transformant la coenzyme I de sa forme réduite (NADH) en celle oxidée (NAD).

L'activité GOT a été calculée en mU/ml plasma séminal.

Le plasma à analyser a été centrifugé (1 ml d'après HILLMANN, 1972) pour 10 minutes à 2-3000 U/minute environ, à l'aide des centrifugeurs à capacité de refroidissement type Janetzky K-24 et VAC-601. Du supernatant ainsi obtenu, 0,100 ml (à des concentrations élevées avec une activité enzymatique intense, 0,020 ml) ont été introduits dans le test suivant: 0,500 solution tampon phosphate, pH=7,6, 0,1 M; 0,220 (0,300) ml aq.dest.; 0,020 NADH (10 mg + 10 mg $NaHCO_3$ ad 1 ml aq.dest.); 0,100 ml ASP (acide aspartique) 100 nM; 0,020 ml MDH/LDH; 0,100 (0,020) ml supernatant. Le début de la réaction enzymatique a été réalisé avec 0,040 ml a-cétoglutarate (40 mMO).

Tous les réactifs utilisés ont été fournis par la firme Boehringer de Mannheim (Allemagne).

Des déterminations de l'activité de cette enzyme ont été effectuées dans les variantes expérimentales suivantes:

1) sperme brut (frais, après 3 heures à la température ambiante normale, après 24 heures à la même température, aprés une congélation immédiate;
2) sperme dilué avec la diluant "8" Hülsenberg (immédiatement après la dilution, après 24 heures à la température du réfrigérateur, idem à un intervalle de 5 jours, après 24 heures à la température du réfrigérateur, puis de congélation;
3) le sédiment dilué avec TG-1 et TG-2 (des diluants pour la congélation, d'après WESTENDORF *et al.*, 1975); (immédiatement après la dilution, après une heure à 5^0C, après 2 heures à 5^0C, après 24 heures a 7^0 C, même opération suivie de congélation).

3. LES RÉSULTATS OBTENUS

Les valeurs de l'activité GOT (exprimées en mU/ml plasma séminal) de toutes les variantes expérimentales sont inscrites dans le Tableau 1.

Afin de mieux marquer les différences, on a construit des graphiques pour chaque variante séparément (Fig.1, 2 et 3).

Les résultats enregistrés mettent clairement en évidence le fait que les trois diluants utilisés n'ont pas d'effets nuisibles sur la membrane du spermatozoïde, les valeurs GOT étant relativement proches.

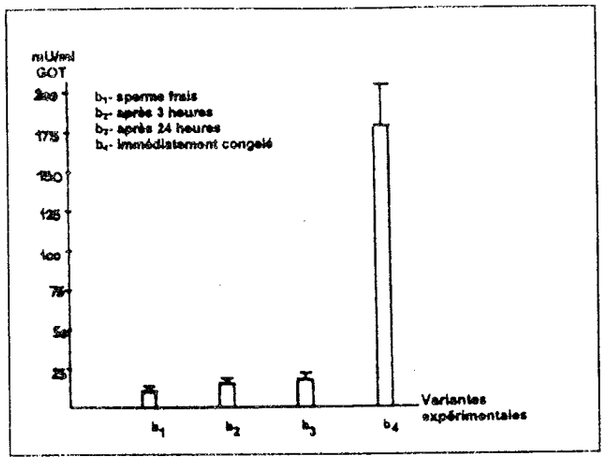


Fig.1 -- L'activité GOT chez le sperme brut.

Tableau 1

L'ACTIVITÉ GOT				
Type de sperme	Sous-variantes expérimentales	Température intermédiaire (°C)	Nombre d'exemplaires	Valeur moyenne GOT en mU/ml plasma séminal
Brut	Frais (jusqu'à 1 heure)	20 – 22	6	11,21± 3,81
	Après 3 heures à la température ambiante normale	20 – 22	5	17,68 ± 3,81
	Après 24 heures à la température ambiante normale, puis congelé	20 – 22	4	21,37 ± 4,80
	Immédiatement congelé	15 – 5	5	177,90 ± 25.43
Dilué avec D 8 (:2) HÜLSENBERG	Immédiatement	30 – 35	6	15,58 ± 8,14
	Après 24 heures au réfrigérateur	0 – 5	6	17.48 ± 3.28
	Après 5 jours au réfrigérateur	0 – 5	6	45.45 ± 20.75
	Après 24 heures au réfrigérateur, puis congelé	15 – 5	5	448.00 ± 72.59
DILUTION avec D8 (1:2)	Immédiatement	15 – 5	6	10,40 ± 5.12
	1 heure à 5° C	15 – 5	4	702.83 ± 125,06
SÉDIMENT avec TG_1 et TG_2 (1:3)	2 heures à 5°C	15 – 5	5	300.20 ± 114.15
	24 heures à 7° C	15 – 7	3	80,35 ± 27.10
	24 heures à 7° C, congelé	15 – 7	3	655,5024 ± 170,31

C=congélation en azote liquide.

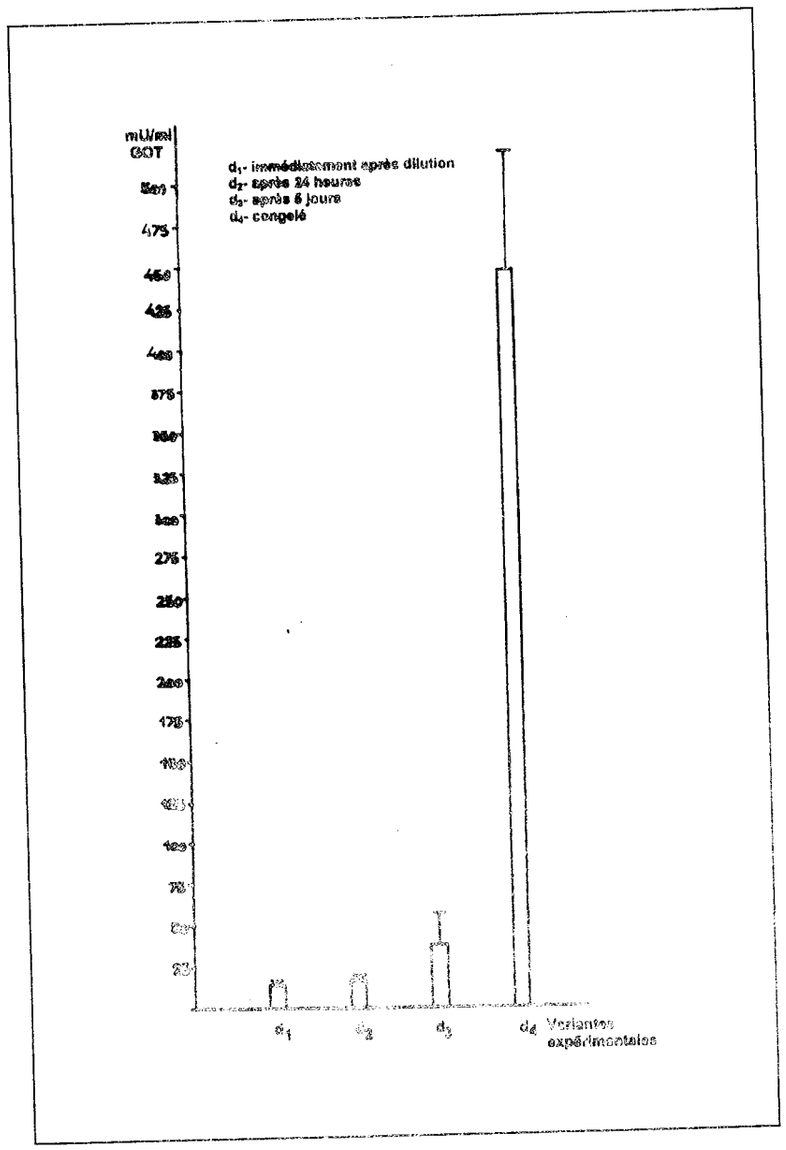


Fig.2 – L'activité GOT chez le sperme dilué avec D_8.

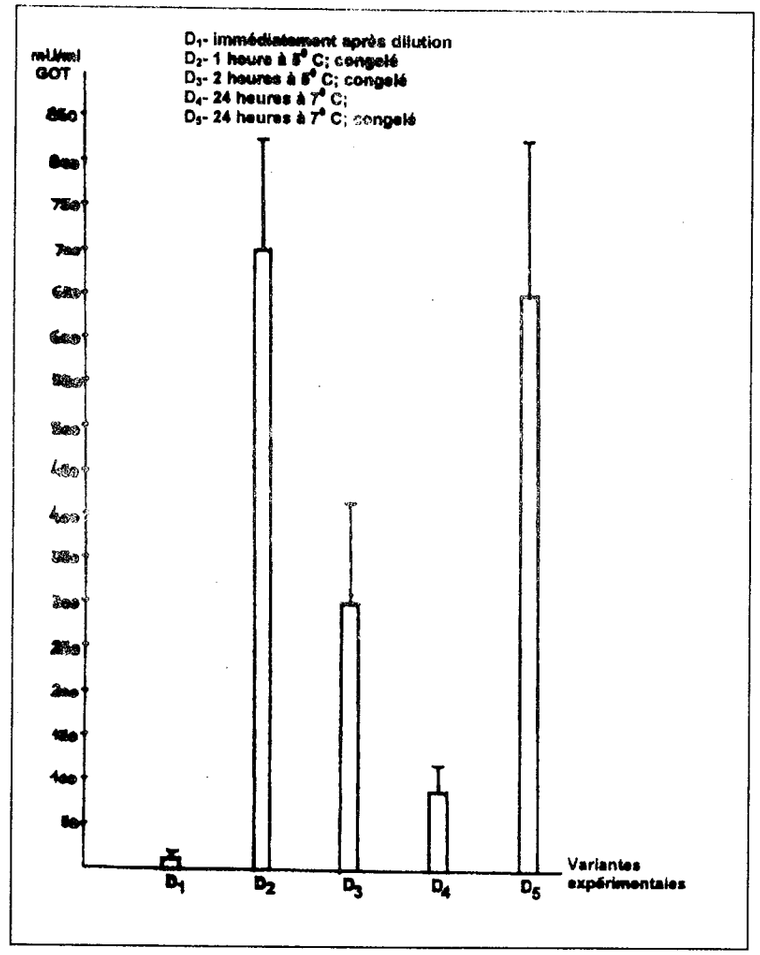


Fig.3 – L'activité GOT chez le sperme dilué avec D_8, TG_1, TG_2.

De même, le fait d'avoir maintenu le sperme dilué pour 1 à 5 jours à la température du réfrigérateur n'engendre pas des valeurs GOT accrues, bien qu'un accroissement gradué puisse être observé. L'étude des trois graphiques nous permet de noter que le processus congélation/décongélation détermine des accroissements marqués de l'activité de cette transaminase. Pourtant, on n'établit pas toujours une corrélation positive entre l'ampleur de cet accroissement et le degré de viabilité des spermatozoïdes, mais seulement entre celle-ci et l'intégrité de la membrane. Ainsi, pour le sperme brut congelé immédiatement (viabilité 0%), la

valeur de l'activité GOT est considérablement plus réduite (177,90 mU/ml plasma) par rapport à celle enregistrée pour le sperme congelé selon le procédé Hülsenberg (300,20 mU/ml plasma), quand la viabilité est présente.

Il est aussi important à noter la bonne corrélation établie entre les modifications du procédé original (Hülsenberg): d'une part, la réduction du temps d'exposition à la température de 5°C (1 heure) et l'accroissement de la température (7°C); d'autre part, l'activité GOT après la décongélation.

4. DISCUSSIONS

Les valeurs de l'activité de cette transaminase, indice de l'intégrité de la membrane du spermatozoide pour le sperme brut (fraîchement récolté, même après 24 heures d'exposition à la température ambiante normale), respectivement, dilué avec les diluants "8", TG-1 et TG-2 montrent un degré modéré d'altération.

La niveau de l'activité GOT (11,21 mU/ml sperme frais et 80,35 mU/ml pour le sperme dilué avant la congélation) est de même ordre de grandeur que les valeurs enregistrées chez le verrat par d'autres auteurs (GRAHAM *et al.*, 1971; HILLMANN, 1972; HILLMANN et TREU, 1973).

Après la congélation, pour toutes les trois variantes (sperme brut, sperme dilué avec le diluant Hülsenberg, sperme dilué avec les diluants "8", TG-1 et TG-2 on constate un accroissement marqué de l'activité enzymatique. Ce résultat concorde avec les conclusions des autres auteurs (HILLMANN, 1972; HILLMANN et BADER, 1973).

Vu que les valeurs les plus basses après la décongélation ont été enregistrées sur les échantillons ayant des pourcentages meilleurs de la mobilité des spermatozoïdes (300,20 mU/ml pour 2 heures de préservation à la température de 5°C), et que dans tous les autres cas les valeurs GOT sont sensiblement plus élevées, nous pensons qu'il y a une corrélation positive entre l'intensité de l'activité de cette enzyme et le degré d'altération de la membrane des spermatozoïdes, causée par l'action stressante des facteurs qui agissent durant le processus de congélation-décongélation.

Nos résultats confirment les conclusions formulées antérieurement par HILLMANN (1972), cependant seulement pour des pourcentages as de mobilité (inférieurs à 5%).

Il revient à la recherche future d'établir si le stress de congélation-décongélation a uniquement l'effet d'altération de l'intégrité de la membrane (entraînant la libération en extérieur de certaines composantes intra-cellulaires, par exemple de certaines enzymes, dans notre cas GOT) ou bien ce stress cause également de profondes transformations d'ordre psychologique.

5. CONCLUSIONS

Une corrélation positive a été établie entre le degré d'altération des spermatozoïdes (de leur membrane cellulaire) consécutive au processus de congélation-décongélation et l'intensité de l'activité GOT déterminée dans le plasma séminal..

L'utilisation de cet indice (l'activité transaminasique) a confirmé l'absence des influences nocives des diluants Hülsenberg utilisés à la préparation des spermatozoïdes pour la congélation.

Nous proposons l'utilisation de cet indice pour l'évaluation de la qualité du sperme de verrat, surtout dans les cas ou apparaissent des changements fonctionnels indécelables par des analyses courantes avec le microscope optique.

BIBLIOGRAPHIE

1. Bergmeyer H.U. et E. Bernt, Methoden der enzymatischen Analyse, 2. Aufl. Verlag Chemie Weinheim, 685–710, 1970.
2. Crabo B.G., S. Einarsson, Acta Vet.Scand., **12**, 125–127 (1971).
3. Flipse R.J., Dvenzal, Sci., **43**, 773–776 (1960).
4. Graham E.F., A.H.J. Rayamannan, M.K.L. Schmehl, M. Maki- Laurila, R.E. Bower, Digest, **19**, *1*, 12–14 (1971).
5. Hillmann K.H., Untersuchungen zur Tiefgefrierkonservierung von Ebersamen mit Beltsville-F3-Verdunner, Disseration, Tierartzl.Hochschule Hannover (1972).
6. Hillmann K.H., H. Bader, Zuchthyg., **8**, 15–21 (1973).
7. Hillmann K.H., H. Treu, Zuchthyg., **8**, 105–112 (1973).
8. Karmen A., J.Clin.Invest., **34**, 131 (1955).
9. Marinescu Al.G., Dorina Marinescu, I. Arisanu, T. Lorintz, Lucr.st. SCCCP Periş II, Buc., 187–195 (1977).
10. Pace M.M., E.F.Graham, Biol. of Reprod., **3**, 140–146 (1970).
11. Polge C., 21, Intern.Fachtagung fur kunstl.Bes. der Haustiere, Wells, Osterreich.
12. Saacke R.G., C.E. Marshall, J.Reprod.Fert., 16, 511 (1968).
13. Westendorf P., L. Richter, H. Treu, Dtsch.Tierarztl.Wschr., **82**, 261–267 (1975).

Reçu le 2 septembre 1999.

[1]*Institut de Biologie, Bucarest, Académie Roumaine*
Splaiul Independenţei 296
[2]*Université de Médicine et Pharmacie, Bucarest*
[3]*Université de Bucarest, Faculté de Biologie*
[4]*Boursier de la Fondation Alex. von Humboldt (Allemagne)*

INCORPORATION OF VITELLIN-GOLD BY OVARIAN FOLLICLES OF *CARASSIUS AURATUS GIBELIO* II. *IN VIVO* STUDIES

OTILIA ZĂRNESCU

Electron microscopy has been used to examine the crucian carp early vitellogenic follicles, *in vivo*, in the presence of electron-dense tracer.

Vitellin was adsorbed to colloidal gold, and the organelles traversed by internalized ligand were examined at 24 hours after intraperitoneal administration.

The gold particles appeared in transport compartments (endosomes, multivesicular bodies) and accumulation compartments (primordial yolk globules).

Moreover, the heterologous yolk protein (chicken vitellin) was internalized by crucian carp oocytes and integrated in yolk globules.

1. INTRODUCTION

Accumulation of yolk protein in oocytes of oviparous vertebrates is an important aspect of oogenesis because these proteins serve as a food reserve for future embryonic development. Yolk proteins are derived from a maternal serum protein, vitellogenin, that is internalized by the growing oocyte through receptor-mediated endocytosis, partially processed and stored in specialized organelles, the yolk granules (7).

In vivo, endocytosis in fish oocyte was studied by quantitative methods (4,8,9,10), light microscopy (3,4,12,13,14) and electron microscopy, with tracers for fluid phase endocytosis (1,12). In the past studies, *in vivo* uptake of tracers for receptor mediated endocytosis in the oocyte of lower vertebrates was studied only in *Xenopus laevis* (11).

We have previously observed that heterologous yolk protein (chicken vitellin) was endocytated by crucian carp oocytes *in vitro* (15).

In the present study we describe, at electron microscopic level, *in vivo* internalization of an electron-dense tracer (vitellin adsorbed with colloidal gold), in the early vitellogenic follicle of crucian carp, *Carassius auratus gibelio*.

2. MATERIALS AND METHODS

Animals: The experiments were carried out on crucian carp. *Carassius auratus gibelio*, obtained from Fisheries Research Station, Nucet. The specimens were acclimatized to laboratory conditions for two or more weeks, at room temperature, in glass aquarium containing tap water.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 44, N° 2, p. 159–164, BUCAREST, 1999

***Preparation of tracer-vitellin gold complex*:** Vitellin (from chicken egg, Sigma) was adsorbed to 15 nm colloidal gold prepared by the tannic acid procedure of Slot and Geuze (5) using the protocol for protein adsorption described by these authors.

Uptake of vitellin-gold in vivo: Each fish was injected intraperitoneally with a colloidal vitellin-gold complex diluted 1:4 with 0.8% saline. Animals were kept in aquarium at 20°C and sacrificed at 24 hours after a single injection.

Electron microscopy : Ovarian follicles were fixed in 2.5% glutaraldehyde in 0.1 M Na-cacodylate, pH 7.4 with 1% dimethyl sulfoxide (DMSO), overnight, at 4°C, washing in the same buffer, overnight at 4°C and postfixed overnight in 1% OsO_4, also in cacodylate buffer. The follicles were then rinsed with cacodylate buffer, dehydrated in ethanol and embedded in Epon 812. Sections were stained with lead citrate and uranyl acetate after sectioning.

3. RESULTS AND DISCUSSION

Early vitellogenic follicles were chosen for *in vivo* observation since these oocytes are in rapid phase of vitellogenesis.

By twenty-four hours after tracer injection, gold particles were present within cortical cytoplasm, in many different compartments, mainly endosomes and multivesicular bodies. There are multiple forms of endosomes, heterogeneous in shape (tubular or vesicular) and the tracers were found at the periphery of these organelles (fig. 1). Multivesicular bodies had a dense interior core with an electron-lucent space surrounding it. The electron-moderate material adhered to luminal surface of the limiting membrane. In the multivesicular bodies that contain condensing yolk protein, gold particles were observed close to the membrane (fig. 1).

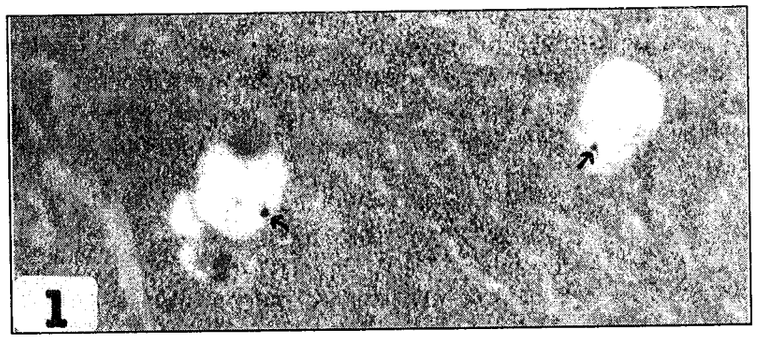
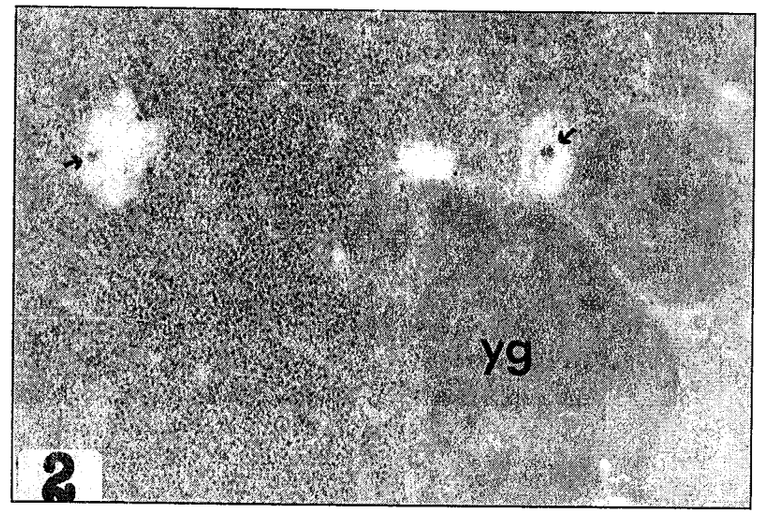
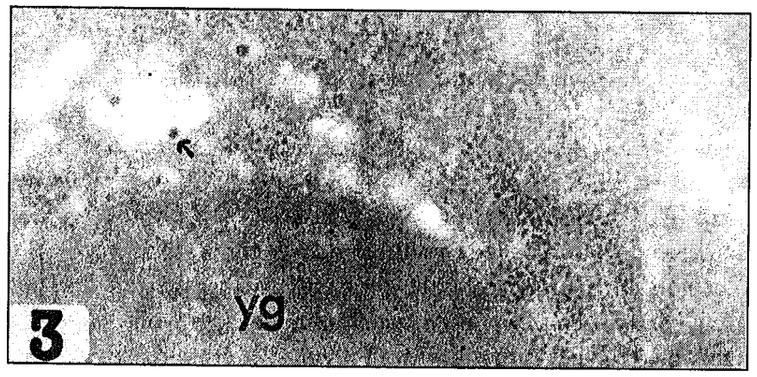


Fig.1 – In the cortical and subcortical cytoplasm of early vitellogenic oocyte, the vitellin gold was found in multivesicular bodies (arrows) ×30.000.





Figs.2-3 – Vitellin gold (arrow) was observed in multivesicular bodies located near the primordial yolk globules (yg) whose size ranged from 0.3 μm (Fig. 2) to 0.1 μm (Fig. 3) ×30.000.

Moreover, the vitellin gold was detected in those endosome/multivesicular bodies near the primordial yolk globules, whose size ranged between 0.3 μm (fig. 2) and 0.1μm (fig. 3).

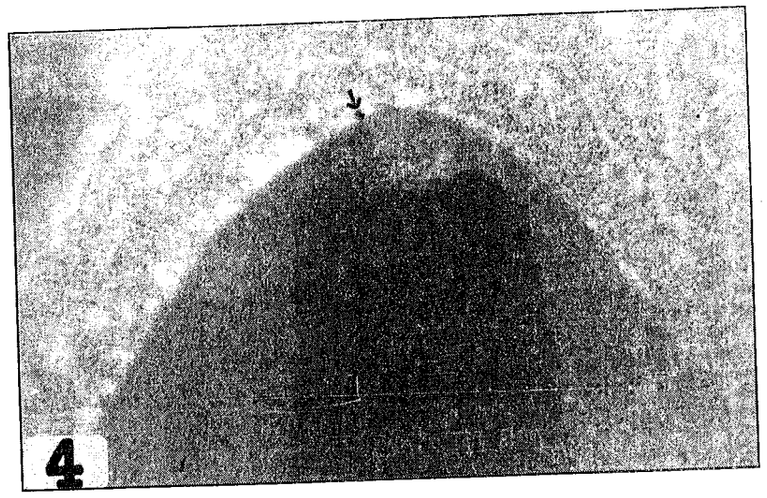


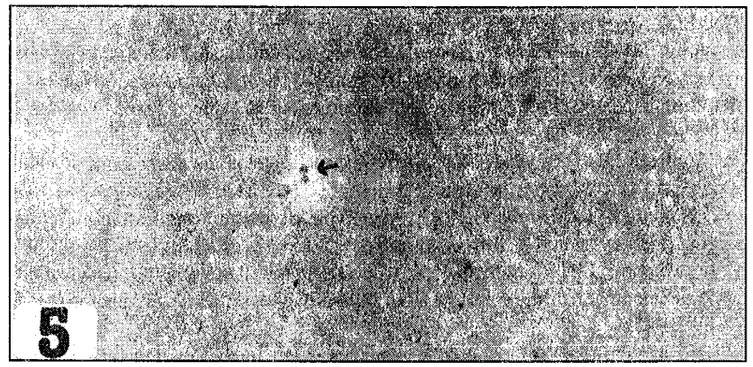
Fig.4 – Tracer also seen at the periphery of mature yolk globules (arrow) ×30.000.

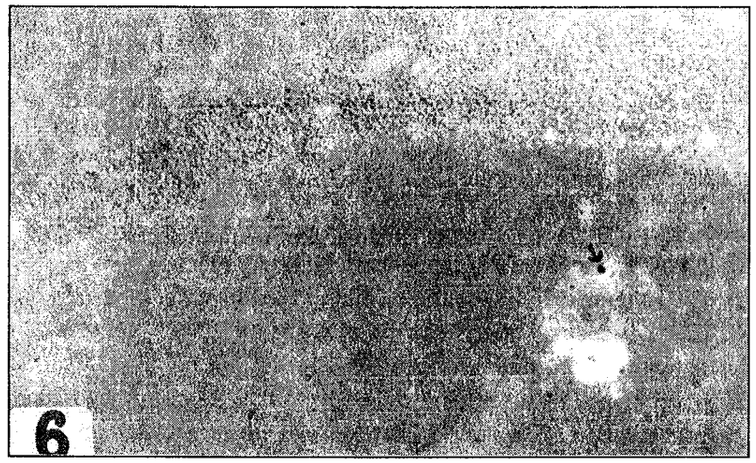
Also, tracer was localized near the membrane of mature yolk globules (fig. 4). Vitellin-gold was observed in larger primordial yolk globules (fig. 5) and in the superficial zone of mature yolk globules, where there are vesicles 0.25 μm in size. These vesicles contain an electron dense tracer (fig. 6).

The number of gold particles encountered within the endocytic compartments of oocytes from animals administered a single tracer injection was low. This might have occurred because tracer-contained vesicles fused with tracer-free vesicles, thus diluting the number of gold particles. Previously, *in vitro* studies on crucian carp oocyte (15) indicated that oocyte endocytosis is constitutive and oocyte receptors are internalized at the same rate whether they are occupied or not.

In our experiments we used vitellin, a chicken yolk protein that was internalized by crucian carp oocytes. Previously, cross-reactivity experiments demonstrated binding of purified chicken and *Xenopus* yolk protein (lipovitellin) to the salmon vitellogenin receptor, verifying that common functional elements of yolk proteins and its receptor have been conserved during vertebrate evolution (2). Moreover, vitellogenin receptor antibodies recognize both *Xenopus* and coho salmon vitellogenin receptors (6).

In conclusion we used an electron-dense tracer for the morphological characterization of *in vivo* endocytosis in the early vitellogenic follicle of the crucian carp. The heterologous yolk protein (chicken vitellin) was internalized by crucian carp oocytes.





Figs.5-6 – Incorporation of gold-tracer (arrows) was observed in larger primordial yolk globules (Fig. 5) and in the superficial zone of mature yolk globules (Fig.6) ×30.000.

REFERENCES

1. Busson-Mabillot S., Endosomes transfer yolk proteins to lysosomes in the vitellogenic oocyte of the trout. Biol. Cell, **51**, 53–66 (1984).
2. Schneider W.J., Vitellogenin receptors: Oocyte-specific members of the low-density lipoprotein receptor supergene family. Int. Rev. Cytol., **166**, 103–137 (1996).
3. Selman K., Wallace R.A., Oocyte growth in the sheepshead minnow: uptake of exogenous proteins by vitellogenic oocytes. Tissue & Cell, **14**, 555–571 (1982).
4. Selman K., Wallace R., Oogenesis in Fundulus heteroclitus. III. Vitellogenesis. J. Exp. Zool., **226**, 441–457 (1983).

5. Slot J.W., Geuze H.J., A new method of preparing gold probes for multiple-labeling cytochemistry. Eur. J. Cell Biol., **38**, 87–93 (1985).
6. Stifani S., Nimpf J., Schneider W.J., A single chicken oocyte plasma membrane protein mediates uptake of very low density lipoprotein and vitellogenin. J. Biol. Chem., **265**, 882–888 (1990).
7. Tyler C.R., Sumpter J.P., Oocyte growth and development in teleost. Fish. Fisheries Rev., **246**, 171–179 (1996).
8. Tyler C.R., Sumpter J.P., Bromage N.R., In vivo ovarian uptake and processing of vitellogenin in the rainbow trout, Oncorhynchus mykiss. J. Exp. Zool., **246**, 171–179 (1988).
9. Tyler C.R., Sumpter J.P., Bromage N.R., Selectivity of protein sequestration by vitellogenic oocytes of the rainbow trout, Oncorhynchus mykiss. J. Exp. Zool., **248**, 199–206 (1988).
10. Tyler C.R., Sumpter J.P., Campbell P.M., Uptake of vitellogenin into oocytes during early vitellogenic development in the rainbow trout. Oncorhynchus mykiss (Walbaum). J. Fish Biol., **38**, 681–689 (1991).
11. Yoshizaki N., In vivo study of vitellogenin-gold transport in the ovarian follicle and oocyte of Xenopus laevis. Develop. Growth & Differ., **34**, 517–527 (1992).
12. Zărnescu O., Meşter R., Caracterizarea ultrastructurala a transportului moleculelor exogene (peroxidaza) in foliculii ovarieni de Carassius auratus gibelio. St. Cerc. Biol. Biol. Anim., **49**, 13–26 (1997).
13. Zărnescu O., Meşter R., Endocitoza in faza fluida a FITC-Dextran in ovocitele de Carassius auratus gibelio in vivo si in vitro. St. Cerc. Biol. Biol. Anim., **49**, 133–138 (1997).
14. Zărnescu O., Meşter R., Oancea A., Moldovan L., In vivo and in vitro study of chondroitin sulphate uptake and distribution in oocytes of crucian carp, Carassius auratus gibelio. Rev. Roum. Biol. Biol. Anim., **42**, 157–165 (1997).
15. Zărnescu O., Meşter R., Incorporation of vitellin-gold by ovarian follicles of Carassius auratus gibelio. I. In vitro studies. Roum. Biol. Biol. Anim., **44**, 3–13 (1999).

Received September 16, 1999.

Faculty of Biology
Splaiul Independenţei 91–95
Bucharest
E-mail: otilia@ibd.dbio.ro

HEMATOPOIETIC BONE MARROW IN THE EXPERIMENTALLY LEAD POISONED RATS

PAULA PRUNESCU

The effect of the lead intoxication on the peripheral blood and the hematopoietic bone marrow was presented. The reticulocytes percentage in the peripheral blood was ten times more elevated than in the controls. The yellow fat marrow myeloid metaplasia was noted. The reticular cells lesions, the delay of the erythrocytes maturation and the osteoclasts damage were described.

1. INTRODUCTION

The lead presence in the environment, due to certain industrial residues (7, 12), is very noxious for men and animals, the chronic effects of lead poisoning determining the complex disorder known as saturnism.

It was necessary to use different experimental models of laboratory animals lead poisoning to understand the cellular reactivity in the damaged organs.

One of the first pathologic effects, following the absorption or the inhalation of the lead (18), was the appearance of severe modifications in the morphology of the blood cells and red bone marrow (2, 9, 11). The lead interferes with the pathway of the hem biosynthesis inhibiting the activity of some of the hem biosynthetic enzymes (1, 5, 7, 18). The effect of the lead action was the rapid occurrence in the blood stream of numerous immature erythrocytes with low hemoglobin concentration (reticulocytes). Other effects of the lead poisoning were the premature degradation of the damaged erythrocytes (1) and the impairment of the red bone marrow activity due to important cellular lesions (14, 15). All these events will co-operate to the development of an important anemia during the chronic lead intoxication.

The present paper studied the red blood cells and the hematopoietic bone marrow morphology and ultrastructure, in a lead poisoning model on the rat.

2. MATERIAL AND METHODS

Animals; treatment. 12 young male Wistar rats, of 65±5g received 6–10 intraperitoneal (ip) inoculations with 1% lead acetate solution in saline. The animals received 5 mg lead acetate/dose to the final loading of 50 mg lead/animal. The sacrification was performed after 48 h from the 6th and the 10th inoculation.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 44, N^{O} 2, p. 165–173, BUCAREST, 1999

Histology. Small fragments of the red bone marrow collected from the femur were fixed in 10% formalin in saline. After the histologic routine processing for the paraffin embedding, the histologic sections of 5 μm thickness were stained with hemalum Mayer-eosin (H.E.) and Giemsa McNamara (G) (16).

Cytology. Blood smears and the red marrow imprints stained with May Grünwald – Giemsa (MG-G) were used.

To observe and count the reticulocytes and to establish their maturation classes (10), the intravital stain with blue brillant cresyl (BBC) was performed.

Electron microscopy. Small fragments of red bone marrow were fixed in 2.5% glutaraldehyde in cacodylate buffer pH 7.2 during 12 h, at 4°C and then postfixed in 1.33% Osmium tetraoxide solution in the same buffer for 2 h, in the darkness and at the room temperature. The samples were processed through the routine methods, for washing, dehydration and embedding in Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in the transmission electron microscopy (TEM).

3. RESULTS

The intravitally stained cytological preparations revealed the high frequency of reticulocytes in the blood (Fig. 1). They were about ten-fold more numerous comparatively with the controls (Table 1).

Table 1

The reticulocytes rate per cent erythrocytes (blood smears)

Animals	RETICULOCYTES %				
	Class 1	Class 2	Class 3	Class 4	TOTAL
Experiment *	5.2 (2 – 7)	6.3 (4 – 8)	5.6 (4 – 7)	1.8 (0.8 – 3.6)	18.7 (10 – 25)
Control	0	0	0	1.6 (0.5 – 1.8)	1.6 (0.5 – 1.8)

* Rats sacrificed at 48 h after 6 lead acetate inoculations.

Striking modifications of the blood cells morphology and percentage were observed in the peripheral blood smears stained with MG – G. The hypochromic red cells, the target red cells ("Mexican hat" erythrocytes) and the erythrocytes with basophilic granules were frequently observed. The cellular composition of the peripheral blood was also altered. The presence of the orthochromatic erythroblasts, of the immature granulocytes and of the great lymphocytes was noticed. The neutrophil granulocytes were less than in the controls. The eosinophil granulocytes were present in the peripheral blood, in a higher percentage (till 10%) comparatively to the controls. Also, a certain cellular fragility of the blood cells was noted.

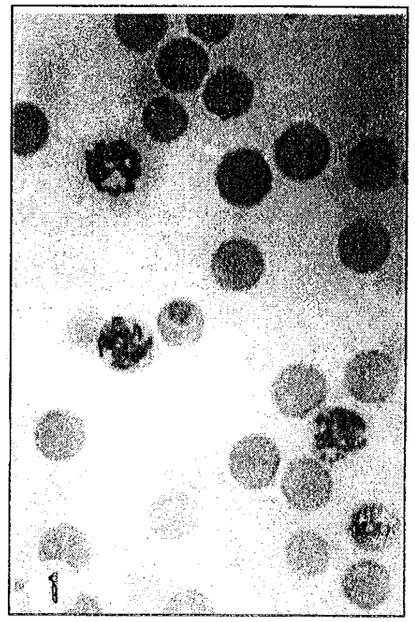


Fig. 1 – Reticulocytes of the maturation classes 1 and 2.
Blood smear, BBC intravital stain (1,600×).

Fig. 2 – Eosinophil and myelocytes; erythroblasts of different stages. Red marrow imprints, MG–G stain (1,600×).

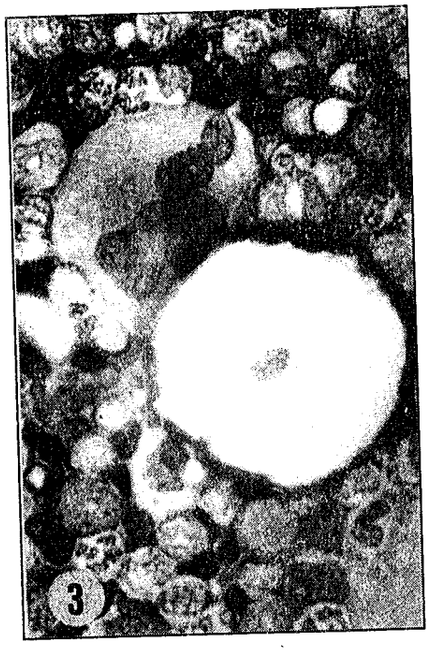


Fig. 3 – The granulocytes outnumbered the erythroblasts in the ratio of 3 or 4 to 1, like in the controls. Note a megakaryocyte and an adipocyte with a central nucleus. Red marrow imprint, MG–G stain (1,200×).

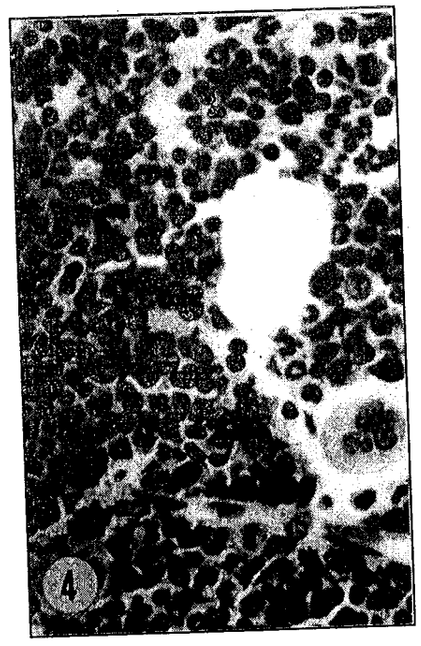


Fig. 4 – Zone of active hematopoiesis. Histologic section, H.E. stain (640×).

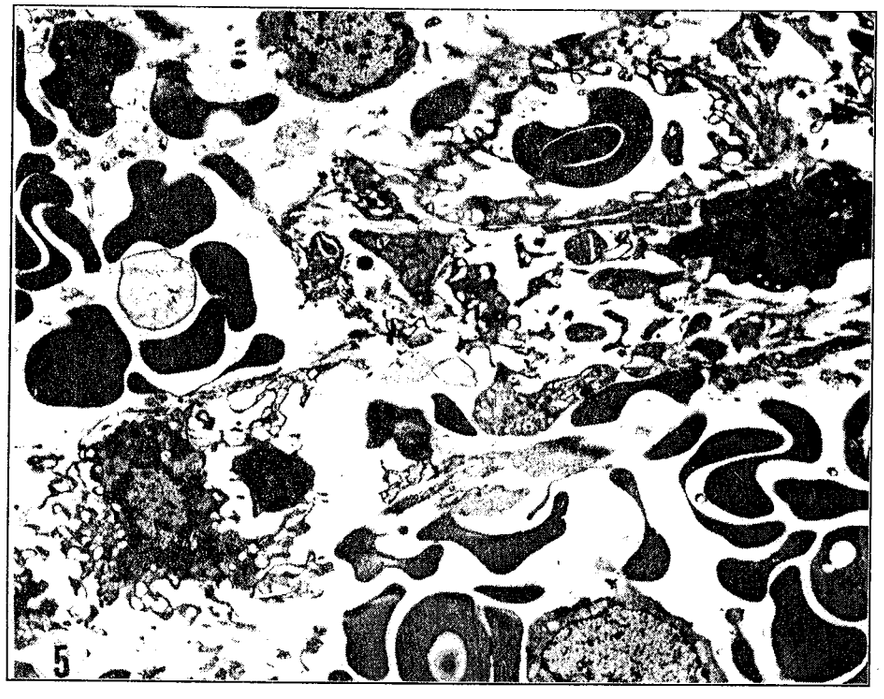


Fig. 5 – Red marrow damaged sinusoids, reticular cells with fine processes and dense cytoplasm, erythroblasts and red cells with severe cellular defects (3,800×).

On the histologic sections, the red marrow presented an active erythropoiesis. The red marrow extended towards the fat yellow marrow areas. The erythroblasts presented a discrepancy between the maturation degree of the nucleus comparatively with the cytoplasm. The degeneration of some erythroblasts was evinced (Figs. 2 and 3). Large adipocytes in the red marrow were frequently observed (Figs. 3 and 4). Like in controls, the granulocytopoiesis obviously surpassed the erythropoiesis (Figs. 2–4). The remarkable increase of the number of immature eosinophil granulocytes was noted (Fig. 2). The incidence of megakaryocytes was high. They showed great heterochromatic areas in the characteristic polylobulated nuclei and a pale voluminous cytoplasm (Figs. 3 and 4).

The noxious effect of the lead treatment was reflected in the fine structure of the hematopoietic cells. The reticular connective cells of the red marrow presented near the nucleus rarefied areas of cytoplasm and long fine peripheral processes (Fig. 5). Some reticular cells were necrosed. The erythrocytes presented numerous small vacuoles. Sometimes, the centre of the red cellular disk became thin (Fig. 5). Some erythrocytes presented fine peripheral processes and numerous small

electrondense bodies (Fig. 5). The preservation of the cellular organelles (mitochondria, polyribosomes, clear vacuoles, autolysomes) and the slight hemoglobin loading of the cytoplasm stroma (Fig. 6) represented the evidence of the maturation delay during the erythrocytes differentiation.

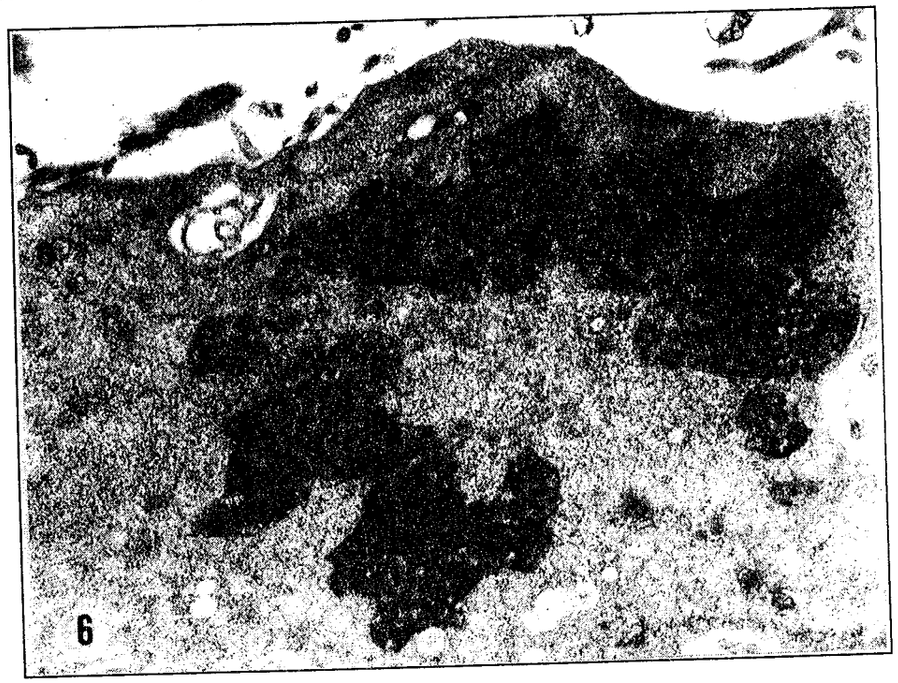


Fig. 6 – Erythroblast division. Note the cytoplasm with poor hemoglobin loading, autolysomes and a multitude of organelles (14,200×).

The megakaryocytes presented great masses of chromatin in the nuclei and a large, dense cytoplasm. The platelets splitting deeply near the megakaryocyte nuclei (Fig. 7) suggested an acceleration in the maturation process of this cellular compartment.

The presence of plasmocytes in the red marrow of the rats treated with lead acetate was frequently noted. In the red marrow of the controls, this cellular type was exceptional. The plasmocytes presented the well-developed granular endoplasmic reticulum, mitochondria, a large cytocentrum and an eccentric nucleus with the particular configuration "in cartwheel". In some plasmocytes fine granular, circular or ovoid-shaped nuclear zones were noticed.

The osteoclasts presented the electrondense nuclei and the coarse cytoplasm (Fig. 8).

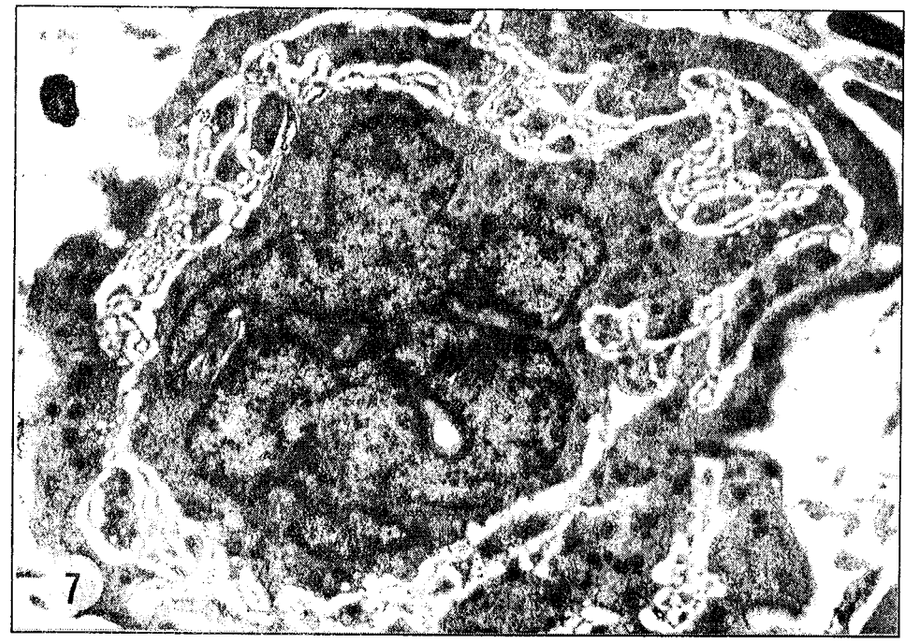


Fig. 7 – The platelets splitting in the damaged megakaryocyte (11,200×).

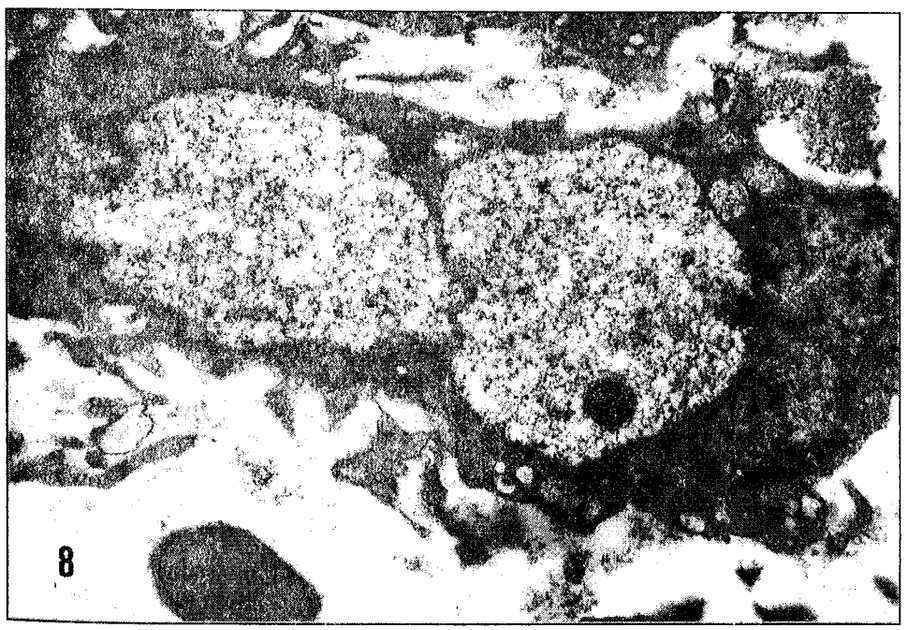


Fig. 8 – Affected osteoclast presenting two great vacuoles with coarse granular material between two nuclei (18,200×).

4. DISCUSSION

The data, obtained during the investigations using laboratory animals, confirmed the value of the experimental models for the understanding of the cellular lesions produced by lead in animals and men. The additional arguments for using the laboratory animals as working models (in the present case, the rat), are the great resistance to the infections of this rodent species, and its easy handling and care. The realization of the lead overloading model of the rat is not expensive or difficult. One can obtain acute or chronic lead poisoning, depending on the amount of lead acetate inoculated and of the length of time after the treatment ceasing. In this way, the pathologic effects of the lead could be considered similar to those known in human (saturnism) (7, 18), to those described in natural poisoned domestic animals (cattle) (12, 13, 14), as well as to those occurring in the waterfowl exposed to lead (1).

After the lead treatment, the circulating red cells complete maturation was rarely encountered. The presence in the peripheral blood of the reticulocytes of the 1, 2, and 3 classes of maturation (Table 1) indicated a red marrow disorder involving red cells differentiation. It was demonstrated that the lead effect on the hem-synthetase enzyme activity takes place late during the erythrocyte differentiation, towards the reticulocyte stage (5, 19).

At the same time, the presence of the immature blood cells in the peripheral blood was considered as the evidence of the blood-red bone marrow barrier dysfunction. The cellular damages observed in the fine structure of the reticular cells and in the endothelial lining of the red marrow sinusoids might be responsible for the impairment of the controlling function of the blood-red marrow barrier. The mature blood cells releasing control by the blood-red marrow barrier was studied for different conditions. The effect of the systemic humoral factors on the pores size of the endothelial lining in the red marrow sinusoids was demonstrated (6, 8, 11).

The insufficient hemoglobin saturation and other features of immaturity representing erythrocytes defects led to the rapid elimination of these cells from the circulating blood. This process was realized by the phagocytosis performed by macrophages of the liver and the spleen. Consequently, the anemia occurred and the red marrow erythropoietic function was considered as inefficient. The red marrow effort to equilibrate the cellular loss was expressed in the process of myeloid metaplasia of the fat yellow marrow.

The osteoclasts and osteoblasts, both implied in the bone formation and remodeling and in the calcium metabolism (3, 4, 17), presented significant ultrastructural damages of the nuclei and cytoplasm, following the lead treatment.

The well-known disorder of the bone metabolism in saturnism (14, 15), consisting in the substitution of calcium by the lead ions, might be ascribed to the damages observed in the fine structure of the osteoclasts.

REFERENCES

1. Amiard-Triquet C., Pain D., Mauvais G., Pinault L., 1992, *Lead Poisoning in Waterfowl* in: *Impact of Heavy Metals on the Environment*, J-P. Vernet ed., Elsevier, Amsterdam, London, Tokyo, New York, 219–245.
2. Angle C. R., McIntyre M. S., Brunk G., 1977, J. Toxicol. Environ. Health, 3 (*3*): 557–564.
3. Ash P., Loutit J. F., Townsen D., 1980, Nature (Lond), 283 (5748): 669–670.
4. Auerbach G. D., Marx S. J., Spiegel A. M., 1992, *Metabolic Bone Diseases* in: Williams, *Textbook of Endocrinology*, J.D.Wilson, D.W.Foster eds., W.B. Saunders Co. Philadelphia, Toronto, Montreal, 1477–1517.
5. Campbell B. C., Brodie M. J., Thompson G. C., Meredith P. A., Moore M. R.,Goldberg A., 1977, Clin. Sci. Mol. Med., **53** (*4*): 335–340.
6. Chamberlain J. K., Weiss L., Weed R. I., 1975, Blood, **46** (*1*), 91–102.
7. Cotrău M., 1978, *Toxicologie - Principii generale*, Ed Junimea, Iaşi, 229 p.
8. Geordano G., Lichtman M. A., , 1973, J. Clin. Invest., **52** (*5*), 1154–1164.
9. Khachirov D.G., Gracheva A. I., 1976, Gir. Tr. Prof. Zabal., *2*, 51–52.
10. Kondi V., 1981, *Laboratorul Clinic de Hematologie*, Ed. Medicală, 463 p.
11. Leblond P. F., Lacelle P. L., Weed R. I., 1971, Blood, **37** (*1*), 40–46.
12. Pinault L., 1985. Symp. Toxicol. Vet., Lyon, 22-23 Avril, 1985.
13. Pinault L., 1988. Rapport d'Études Programme, École. Nat. Vet. Nantes, 1–44.
14. Pinault L., Milhaud G., 1983, *Evaluation of Subclinical Lead Poisoning* in: Pharmacology and Toxicology. I. Ruckenbusch ed., MTP Press, 715–724.
15. Popa I., 1978. *Toxicologie*. Ed. Medicală. Bucureşti, 372 p.
16. Putt F. A., 1972, *Manual of Histopathologic Staining Methods*, John Wiley and Sons, Inc., New York. Toronto. Sydney.
17. Rosenberg A. E., 1994, *Skeletal System and Soft Tissue Tumors (Bones)*, in: Robbins *Pathology Basis of Disease*. R.S.Cotran. V. Kumar, S.L.Robbins eds., W.B. Saunders Co., 1213–1271.
18. Scholmerich J., Ochs A., 1991, *Trace Elements* in: *Oxford Textbook of Clinical Pathology*, vol. 1, M. McIntyre ed., Oxford University Press, 21, 1–220.
19. Verhoeff N. J. P., Noordeloos P. J., Leijnse B., 1978, Clin. Chim. Acta., **82** (1/2), 45–54.

Received July 26, 1999.

Institute of Biology
Splaiul Independenţei 296
Bucharest 79651
P.O. Box 56-53
Romania

STRUCTURAL CHANGES CAUSED BY LEAD ACTION ON THE HEPATOPANCREAS OF *ANODONTA CYGNAEA* L. (MOLLUSCA, LAMELLIBRANCHIATA)

VIORICA MANOLACHE *, VIRGINIA POPESCU-MARINESCU **,
MARIA NĂSTĂSESCU *, DANIELA TEODORESCU**, ELENA NEAGU **

Upon the action of 210, 289, 771 µg/l Pb concentrations for different periods of time, i.e. 24 hours, 72 hours, 7 days, a series of structural changes occurred at hepatopancreas level in *Anodonta cygnaea*, most of them of irreversible character. Hepatopancreas structural degradation was, generally, in direct relation to toxicant dose and the time of organisms exposure to its action. Gradual disorganization of hepatopancreas tubules consisted mainly of: their spreading, clear cell vacuolization, toxicant granules' appearance in the cytoplasm of amoebocytes and clear cells, dark cell rarefaction. Finally, upon the influence of 771 µg/l Pb dose for 7 days, even a total disorganization of hepatopancreatic tubules occurred, including that of the connective tissue between them.

1. INTRODUCTION

The hepatopancreas is one of the organs accumulating large amounts of heavy metals and which, at the same time, tolerates them to a certain extent. Beside the heavy metals considered as essential elements (Cd, Mn, Fe, Ni, Cu, Zn), lead is present in different organisms as well. But, so far its role is not well established, either in plants, or in animals. But, in the last decades this element often occurs more and more frequently in some aquatic basins, exceeds the admissible limits. Its origin in the surface waters is usually due to industrial discharges.

In the present work, the structural changes occurring at the level of *Anodonta cygnaea* hepatopancreas upon the action of various lead concentrations were followed. We mention the fact that as concerns the mollusca, there are few researches regarding the lead action upon various tissues. But, both George [2] and Marshall and Talbot [6] refer especially to this element accumulation in various cell categories. The former take into consideration *Ostrea sp.* and *Mytilus sp.* hepatopancreas, the latter, basal lamina forming the capillary walls in gill lamellae of *Mytilus edulis*.

2. MATERIALS AND METHODS

In order to find out the changes induced by lead action (from lead acetate) in the hepatopancreas of *Anodonta cygnaea*, first, the animals' intoxication was performed by the static test method. In this respect, several batches of lamellibranchiata were maintained in lead solutions of 210, 289 and 771 µg/l.

REV. ROUM. BIOL.– BIOL. ANIM., TOME 44, N° 2, p. 175–180, BUCAREST, 1999

At periods of time, namely 24 hours, 72 hours and 7 days some specimens were killed, dissected and portions of hepatopancreas were fixed in Bouin. Further, the material was processed by classic histological techniques and analysed by light microscopy.

3. RESULTS

3.1. TIME OF LEAD ACTION 24 HOURS

3.1.1. *Toxicant dose* 210 µg/l Pb

At this dose, not all the hepatopancreas tubules were affected. In those with destroyed basal membrane, in some areas, disorganization, especially of clear cells, was noticed. These cells had the spherical nucleus with an evident nucleolus and uniformly distributed chromatin in the control, and the same cells, upon the lead action, had altered both the nucleus shape, which became irregular and chromaticity; they appeared pycnotic. In disorganized tubules, the clear cells lost their cell limits as well, and sometime cell fusion was noticed, nuclei appearing scattered. Also, in these clear cells, a series of brownish inclusions were distinguished, representing, probably, lead inclusions. As concerns the dark cells of the hepatopancreas, they were less affected and usually agglomerated into a portion of the tubule. The connective tissue surrounding the hepatopancreas tubules, generally undergoes pronounced alterations. Connective cell nuclei are deformed and pycnotic and amoebocytes are diminished in size and frequently with pycnotic nuclei. Collagen fibres are disorganized as well (Fig.1).

3.1.2. *Toxicant dose* 289 µg/l Pb

At this dose, structurally, tubules appeared both intact and disorganized. The unistratified epithelial arrangement generally remained valid for dark cells, although in these ones, the limits between them sometimes disappeared. Plasmalemma of dark cell apical pole was generally visible. At this intoxication, the clear cells were very altered, the cell limits within them were not distinct; the cells were scatered towards the tubule centre. Nuclei were pycnotic as well. Connective tissue appeared very destroyed, and nuclei of connective tissue and amoebocytes were pycnotic (Figs. 2 and 3).

3.1.3. *Toxicant dose* 771 µg/l Pb

This high dose even much more affected the hepatopancreas tubule cells. In the case of remained relatively intact tubules, the dark cells also appeared agglomerated into a certain portion of tubules. Clear cells, with destroyed nuclei, had no more limits and the cytoplasm got a pronounced granular appearance.

In many tubules, in their centres, a granular mass was also distinct, with a few deformed and pycnotic nuclei. Very many hepatopancreas tubules had their basal membrane disrupted. The connective tissue was so destroyed that very often the collagen fibres were no more distinct. Only the scattered nuclei of connective cells and amoebocytes appeared (Fig. 4).

3.2. TIME OF LEAD ACTION 72 HOURS

3.2.1. *Toxicant dose* 210 µg/l Pb

Hepatopancreas tubules, at this dose, were greatly modified structurally in that most of them appeared with disrupted basement membrane. In some tubules, large vacuoles were distinct in which nuclei remains were present. Cell limits, generally, disappeared and in the tubule centre, the granular mass appeared. The connective tissue between tubules was very rarefied. A few pycnotic nuclei and traces of amoebocytes were also distinct (Fig. 5).

3.2.2. *Toxicant dose* 289 µg/l Pb

Hepatopancreas tubules were strongly affected. Cellular limits, particularly at clear cells, disappeared, their cytoplasm being highly vacuolized. Towards the middle of the tubule, generally, cell remains and pycnotic nuclei were distinct. Dark cells were also noticed to be agglomerated to the periphery, sometimes maintaining their ovoid nuclei. Other times, towards the tubule periphery, spherical nuclei agglomerations were noticed as well. Collagen fibres, at this dose were completely destroyed too and nuclei of various cell categories were degraded (Fig. 6).

3.2.3. *Toxicant dose* 771 µg/l Pb

Most hepatopancreas tubules appeared disrupted and the cell epithelial arrangement was generally lost. Most cells, very rarefied, had a vacuolized cytoplasm. Destroyed cells were agglomerated towards the tubule centre and nuclei were scattered. Connective tissue was greatly modified and connective fibres were strongly affected. Connective cell nuclei were deformed, pycnotic. Also, many amoebocytes were greatly changed structurally (Fig. 7).

3.3. TIME OF LEAD ACTION 7 DAYS

3.3.1. *Toxicant dose* 210 µg/l Pb

At this dose, some tubules appeared with intact basement membrane. In this situation, the confluence of some hepatopancreas tubules was noticed as well. At the level of these formations, other structural changes were observed too. Thus

cell limits disappeared, nuclei were very rarefied and the remaining ones were of very small sizes, deformed and pycnotic. In the centre of some tubules, sometimes an agglomeration of disintegrated cell fragments was noticed. Connective tissue was also modified; collagen fibres were sometimes no more distinct; connective cell nuclei were very rare, deformed and pycnotic and here and there an apparently structurally intact amoebocyte could be noticed as well.

3.3.2. *Toxicant dose* 289 µg/l Pb

Hepatopancreas tubules were very changed. In this situation, the most affected were the clear cells, which appeared destroyed, with lost cell limits. The respective cells were sometimes fused; the cytoplasm of some of them was much vacuolized. The dark cells appeared rarefied, maintaining their location at the tubule periphery. At the centre of the hepatopancreas tubule, a granular mass was distinct. Many tubules were completely disorganized. The connective tissue appeared with the same alterations as at the previous dose (Fig. 8).

3.3.3. *Toxicant dose* 771 µg/l Pb

At this dose, the structural change was very pronounced, hepatopancreas tubules had disorganized cells. Towards the tubule centre, there were distingnished deformed and pycnotic nuclei, or a simple granular mass resulted from the cell disintegration. Connective tissue was completely disorganized too (Fig. 9).

4. DISCUSSION

From our observations as well as from the existing speciality literature it results that the hepatopancreas is the most sensitive organ to pollutant action. Thus, hepatopancreas alterations caused by mercury or copper action were reported by Andersen and Baatrup in *Crangon crangon* crustacean [1], also by Popescu-Marinescu et al. in *Astacus leptodactylus* [9].

In *Mytilus galloprovinciales* and *Ruditapes phillipinaeum* mollusca, Sarasquette et al., following the cadmium and copper intoxications, noticed in the digestive gland a series of degenerative processes [10].

Structural changes in the hepatopancreas of *Anodonta cygnaea* or *Anodonta piscinalis* species, exposed to intoxication with mercury, copper, zinc and ammonium chloride, were described by Manolache et al. [4,5] and Năstăsescu et al. [7,8]. Thus, there were revealed the hepatopancreas tubule degradations, cytoplasm vacuolization, plasma membrane disappearance, the cell spreading within the tubule lumen and destruction of clear cells particularly.

Pronounced degradations were noticed by us too, in *Anodonta cygnaea* species, by lead intoxication, from lead acetate. In this case, beside the above

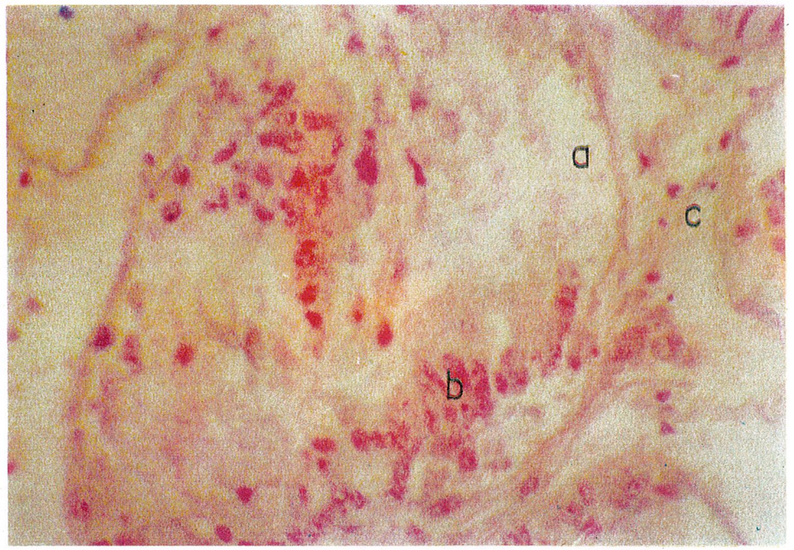


Fig. 1. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 210 µg/l Pb concentration, for 24 hours: a = vacuolized clear cells; b = dark cells; c = connective tissue (40 · 0.65).

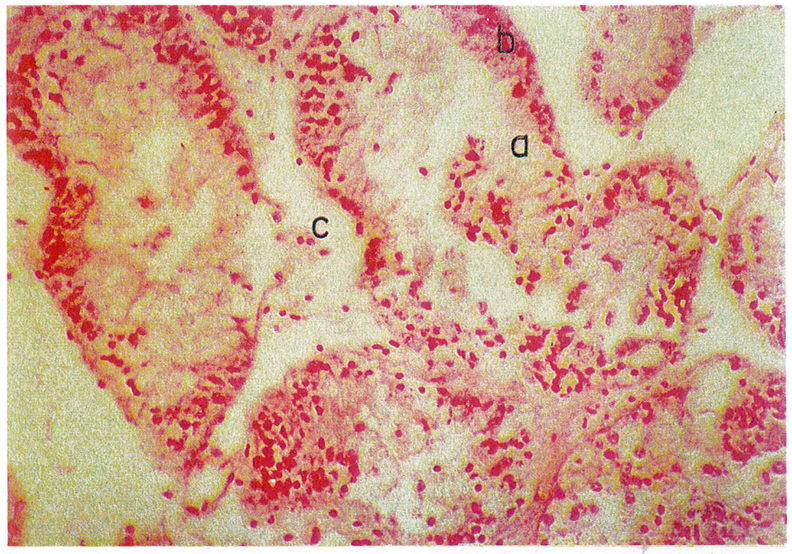


Fig. 2. – Structural changes at the level of *Anodonta cygnaea,* hepatopancreas upon the action of 289 µg/l Pb concentration, for 24 hours: a = clear cells; b = dark cells; c = connective tissue (20 · 0.40).

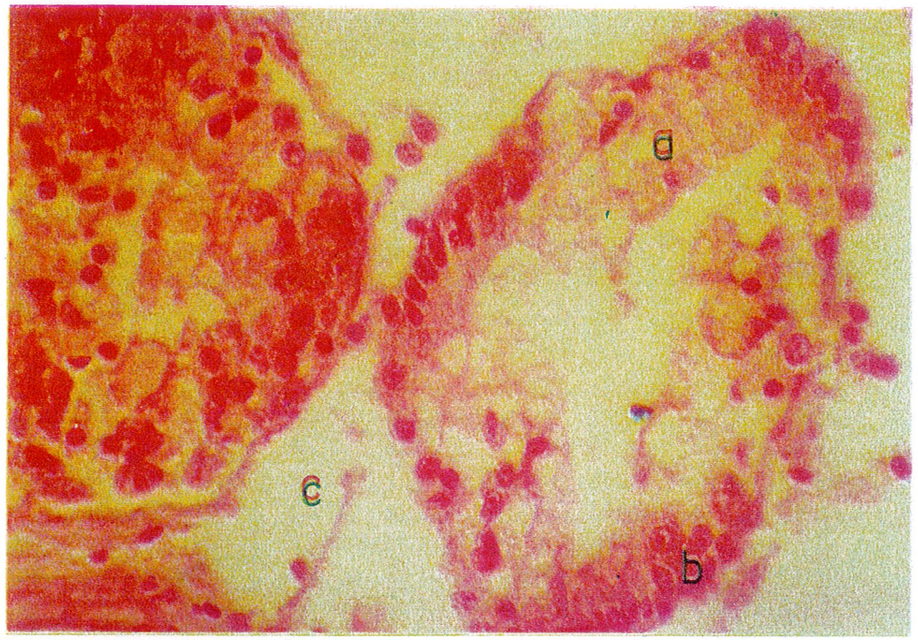


Fig. 3. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 289 µg/l Pb concentration, for 24 hours: a = clear cells; b = dark cells; c = connective tissue (40 · 0.65).

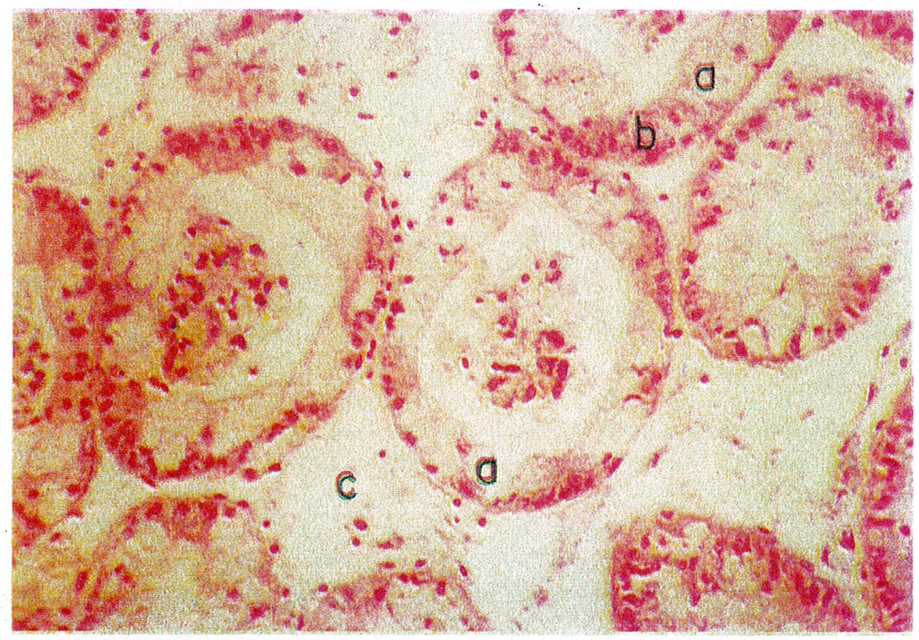


Fig. 4. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 771 µg/l Pb concentration, for 24 hours: a = clear cells; b = dark cells; c = connective tissue (20 · 0.40).

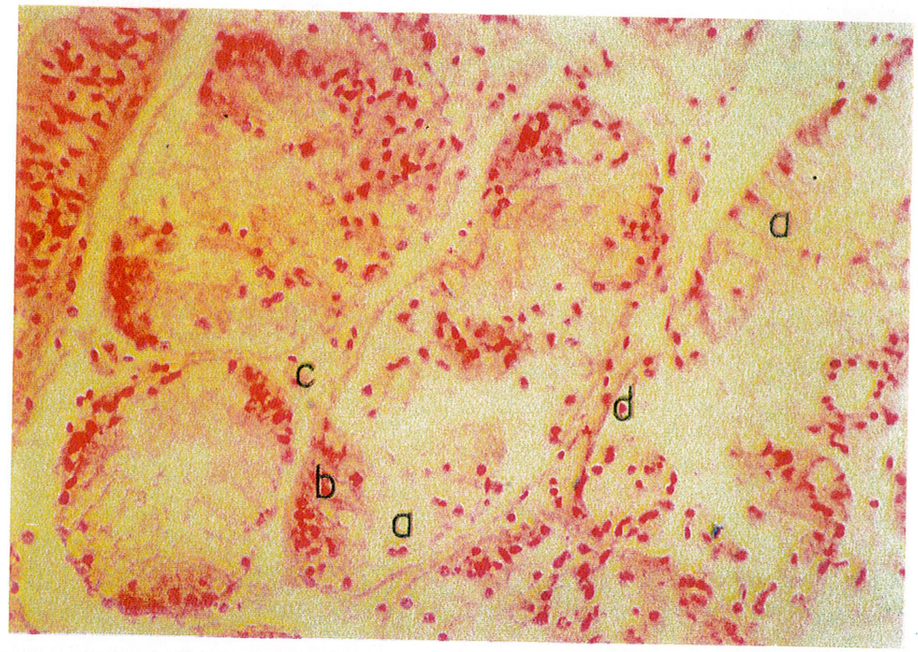


Fig. 5. – Structural changes at the level of *Anodonta cygnaea,* hepatopancreas upon the action of 210 µg/l Pb concentration, for 72 hours: a = vacuolized clear cells; b = dark cells; c = connective tissue; d = destroyed basement membrane (20 · 0.40).

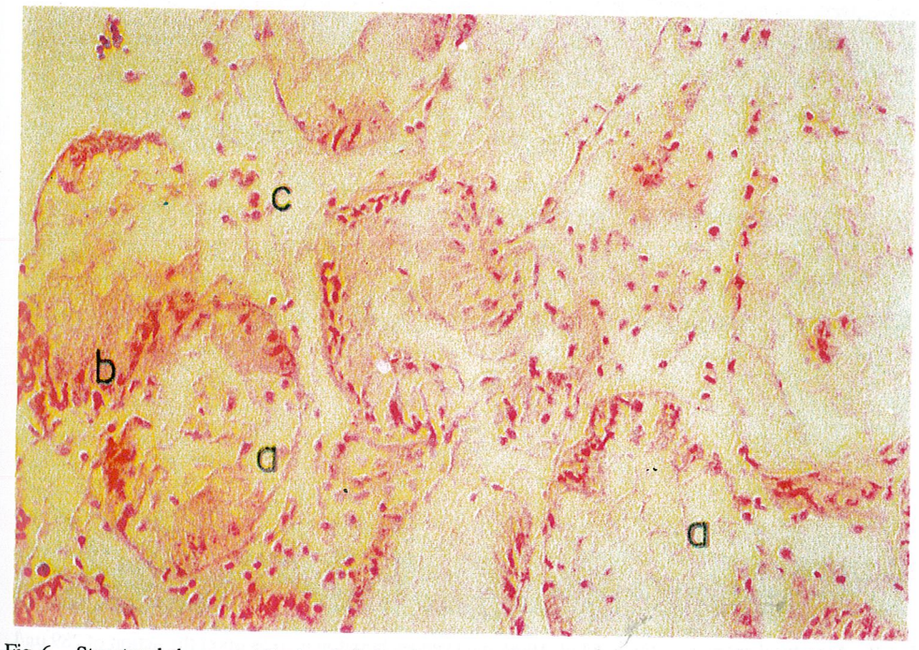


Fig. 6. – Structural changes at the level of *Anodonta cygnaea,* hepatopancreas upon the action of 289 µg/l Pb concentration, for 72 hours: a = clear cells; b = dark cells; c = connective tissue (20 · 0.40).

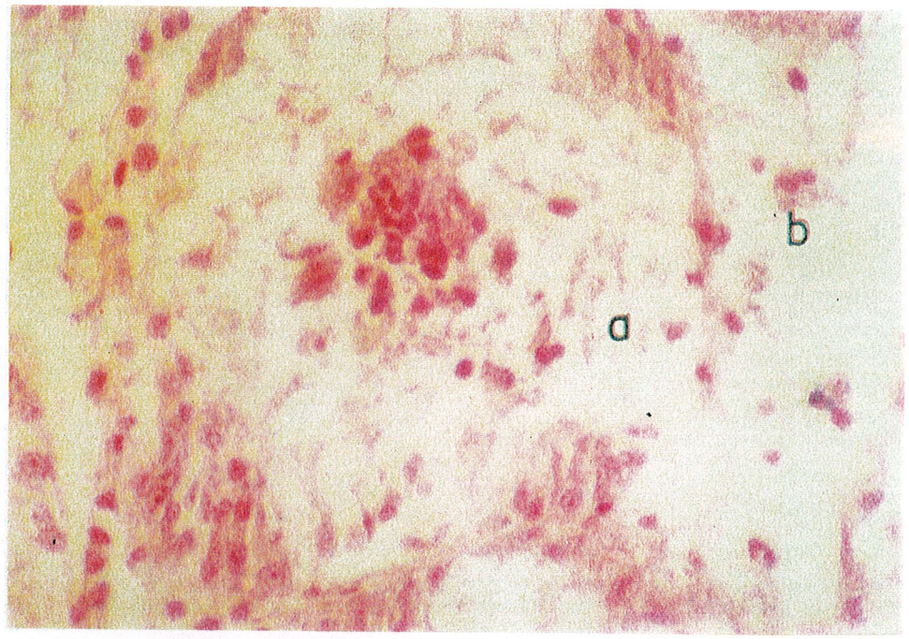


Fig. 7. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 771 µg/l Pb concentration, for 72 hours: a = hepatopancreatic tubule; b = connective tissue (40 · 0.65).

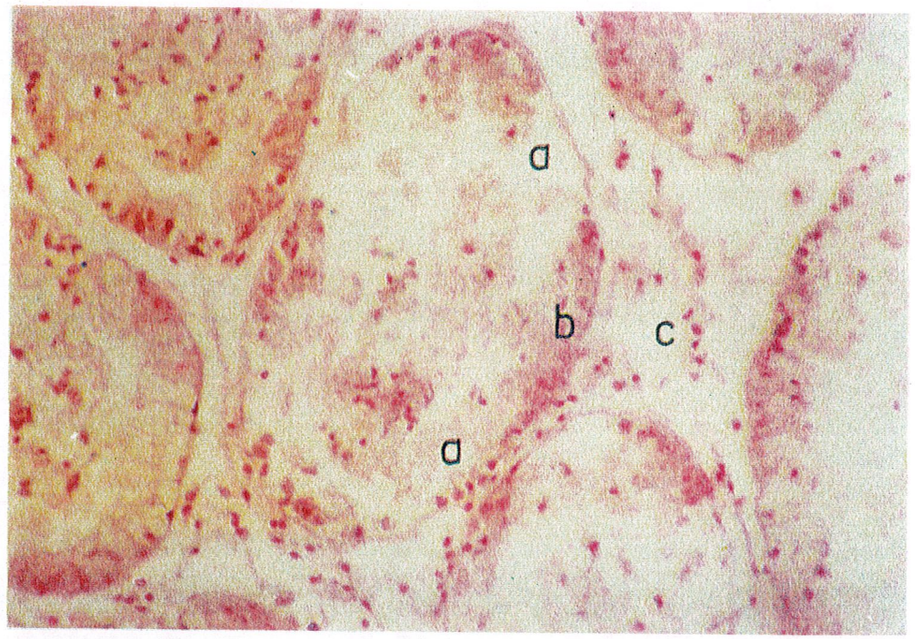


Fig. 8. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 289 µg/l Pb concentration, for 7 days: a = fused clear cells; b = rarefied dark cells; c = connective tissue (40 · 0.65).

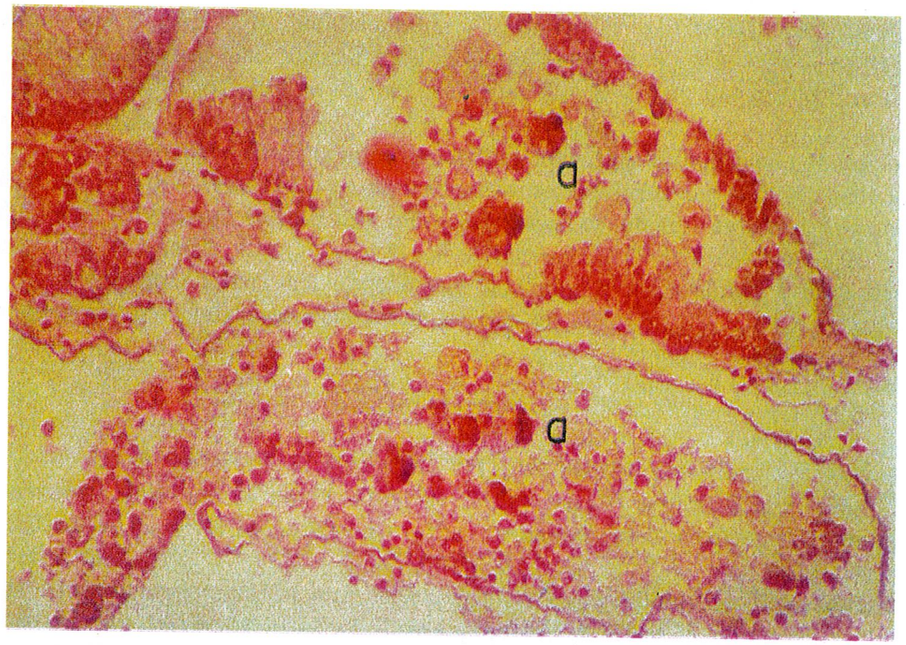


Fig. 9. – Structural changes at the level of *Anodonta cygnaea* hepatopancreas upon the action of 771 µg/l Pb concentration, for 7 days: a = completely disorganized hepatopancreatic tubules (20 · 0.40).

mentioned changes we also observed affection to some extent of the dark cells in tubules, but which maintain their position at its periphery. We also noticed strong degradations of the connective tissue surrounding the hepatopancreas tubules, where both connective cells and collagen fibres were destroyed.

In many invertebrate species, it is described that by metal incorporation, intracellular mineral deposits are formed like some granules surrounded by membranes [3,11,12]. In mollusca, namely in *Ostrea sp.* and *Mytilus sp.*, George noticed copper and lead accumulation in amoebocytes or in other cell categories [2]. The granules reported in various cell types also represent the site of metals' sequestration.

We also noticed such granules' accumulation in *Anodonta cygnaea* in case of lead intoxication. Thus, we have noticed numerous amoebocytes with granules in the connective tissue surrounding the hepatopancreas tubules, as well as in the clear cell cytoplasm.

We outline the fact that amoebocytes belonging to macrophagic system play a major role in metals' bioaccumulation and deposit.

5. CONCLUSIONS

1. Upon the action of 210, 289, 771 μg/l Pb concentrations for various periods of time, i.e. 24 hours, 72 hours, 7 days, a series of structural changes occurred at the level of *Anodonta cygnaea* hepatopancreas, most of them being irreversible.

2. Structural degradation of hepatopancreas was, generally, in direct relation with toxicant dose and time of organism exposure to its action.

3. Gradual disorganization of hepatopancreas tubules consisted mainly of: basement membrane disruption, cell limits' disappearance, nuclei pycnosis and spreading, clear cell vacuolization, the appearance of toxicant granules in the cytoplasm of clear cells and amoebocytes in the connective tissue, rarefaction of dark cells. Finally, it came to the situation when upon the action of 771 μg/l Pb dose for 7 days, even a total disorganization of hepatopancreas tubules occurred, including the connective tissue between them.

REFERENCES

1. Andersen J. T., Baatrup E., *Ultrastructural localization of mercury accumulations in the gills, hepatopancreas, midgut and antennal glands of the brown shrimps Crangon crangon*, Aquatic Toxicology, **13**, 309–324 (1988).
2. George S. G., *Correlation of metal accumulation in mussels with the mechanisms of uptake, metabolism and detoxification*, Thalassia Yugoslavica, **16**, 347–365 (1980).

3. Hopkin P.S., NOTT S. A., *Some observation on concentrically structural intracellular granules in the hepatopancreas of the shore crab Carcinus moenas L.*, J. mar. biol., ASS. U.K., **59**, 867–877 (1979).
4. Manolache Viorica, Năstăsescu Maria, Zărnescu Otilia, *Acţiunea mercurului asupra hepatopancreasului la Anodonta piscinalis (Mollusca, Bivalvia)*, Analele Univ. Buc., **44**, 37–42, Bucureşti (1995).
5. Manolache Viorica, Popescu-Marinescu Virginia, Năstăsescu Maria, Marinescu Carmen, *Structural changes induced by copper intoxication in Anodonta cygnaea L. (Lamellibranchiata) gills and hepatopancreas,* Rev. roum. biol., Biol. anim., **42**, 1, 41–48, Bucarest (1997).
6. Marshall A. I., Talbot V., *Accumulation of cadmium and lead in the gills of Mytilus edulis: X - ray microanalysis and chemical analysis*, Chem. Biol. Interact., **27**, 1, 111–123 (1979).
7. Năstăsescu Maria, Popescu-Marinescu Virginia, Manolache Viorica, Marinescu Carmen, *Acţiunea zincului asupra mantalei, branhiilor şi hepatopancreasului de la Anodonta cygnaea* (Mollusca - Lamellibranchiata), St. cerc. biol., Seria biol. anim., **49**, 2, 147–155, Bucureşti (1997).
8. Năstăsescu Maria, Popescu-Marinescu Virginia, Manolache Viorica, Teodorescu Daniela, *Structural changes caused by ammonium chloride action at the level of hepatopancreas in Anodonta cygnaea L. (Mollusca, Lamellibranchiata),* Rom. J. Biol. Sci., **II**, 1-2, 103–112, Bucharest (1998).
9. Popescu-Marinescu Virginia, Manolache Viorica, Năstăsescu Maria, Marinescu Carmen, *Structural modifications induced by copper in Astacus leptodactylus (Crustacea Decapoda) hepatopancreas*, Rom. J. Biol. Sci., **I**, 1–2, 99–105, Bucharest (1997).
10. Sarasquette M. C., Gonzales M. L., Gimina S., *Comparative histopathological alterations in the digestive gland of marine bivalve exposed to Cu and Cd*, Eur. J. Histochem, **36**, 223–232 (1992).
11. Simina T., Boss J. L., *Structure and function of the calcirne cells of the fresh water pulmonate snail Lymnaea stagnalis*, Netherlands J. of Zool., **27**, 195–208 (1977).
12. Simkiss K., *Biomineralization and detoxification*, Calcified Tissue Research, **24**, 199–200 (1977).

Received June 28, 1999.

*Faculty of Biology,
Spl. Independenţei 93–95,
Bucharest

** National Institute of Research and Development for Biological Sciences
Spl. Independenţei 296,
Bucharest

STRUCTURAL CHANGES CAUSED BY LEAD ACTION ON GILLS OF *ANODONTA CYGNAEA* L. (MOLLUSCA, LAMELLIBRANCHIATA)

VIRGINIA POPESCU-MARINESCU*, MARIA NĂSTĂSESCU**,
VIORICA MANOLACHE**, DANIELA TEODORESCU*, ELENA NEAGU*

Batches of *Anodonta cygnaea* specimens were experimentally exposed to the action of 210, 289 and 771 µg/l lead doses (from lead acetate) during various times, namely: 24 hours, 72 hours, 7 days. Main structural changes at gill level were shown by disorganization or even destruction of gill epithelium. The limits between ciliated and mucous cells disappeared; cytoplasm dispersion as well as nuclei pycnosis occurred. Degradation was also expressed at the level of connective tissue. amoebocytes and also blood sinuses. In the cytoplasm of several cell types. several toxic granules accumulated, including within destroyed sinuses. In general. cell and tissue changes were proportional to the used dose and time of organism exposure to lead action.

1. INTRODUCTION

Our investigations were directed to lead action on *Anodonta cygnaea* gill structure, since this metal, exceeding certain limits, has a high toxicity for aquatic organisms. It is well known the fact that the filtrators, i.e. mollusca sensitivity to this pollutant, is rather high. At the same time, the respective organisms have a particular role in accumulating the lead from water in their bodies; the metal has an extremely low biological degradation rate. On the other hand, many mollusca species have a particular significance from the economic point of view as well.

Most of specialty literature works analysing heavy metal toxicity, including lead, refer especially to the surviving time, lethal doses, the bioaccumulation, the bioconversion, etc. [2,3,4,6,7,11].

Nevertheless, we outline the fact that the action of heavy metals on the structure of different types of tissues and cells, generally in invertebrates, so much the more in mollusca, was less studied [1,5,8,9,10].

2. MATERIALS AND METHODS

The literature data indicate for different aquatic invertebrates doses of 0.1–6 mg/l Pb as being lethal. We have followed this metal action on *Anodonta cygnaea* structure, at concentrations of less than 1 mg/l Pb.

In order to prepare the material required for our investigations, batches of tens of adult specimens of this lamellibranchiate collected from nonpolluted aquatic basins at Cornești Farm, were first acclimatized under aquarium conditions in laboratory and then, by static tests, were exposed to lead influence (from lead acetate) from aqueous solutions at original concentrations of 210, 289 and 771 µg/l. The period of mollusca maintaining in the respective solutions was of maximum 7 days. At 24 hours, 72 hours and 7 days-periods of time some specimens were killed, dissected and portions of external and internal gills were fixed in Bouin. The material prepared in this way was processed by classic histological techniques and analysed by light microscopy.

3. RESULTS

3.1. STRUCTURAL CHANGES AT EXTERNAL GILL LEVEL

3.1.1. Time of lead action 24 hours

3.1.1.1. *Toxicant dose* 210 µg/l Pb

Gill epithelium in many areas was completely disorganized by the disappearance of the limits between ciliated and mucous cells. Cell apical pole was destroyed so that they had the cytoplasm destroyed; only ovoid, pycnotic nuclei could be noticed. In gill axis, the connective tissue was greatly rarefied: both connective cells and amoebocytes appeared degraded (Fig. 1). In some amoebocytes, also nucleus was noticed located at the periphery and appeared flattened and pycnotic. In some cells' cytoplasm, granulations sometimes occurred. Most blood sinuses had a destroyed epithelium, many of them were confluated and some of them were mixed with the connective tissue in gills axis (Fig. 2).

3.1.1.2. *Toxicant dose* 289 µg/l Pb

At this dose, gill epithelium degradation was even more pronounced. In many areas, the epithelium was no more discernible, the connective tissue was not in the gill axis. In areas where the epithelium was still discernible, this had the cells completely destroyed and the connective tissue and the muscular tissue in the gill axis were affected; all the remaining nuclei had a pycnotic appearance. Blood sinuses were greatly dilated or destroyed. Usually instead of connective tissue and blood and connective elements, granular black deposits appeared representing probably the toxic substance; where amoebocytes still occurred, they were either enlarged or deformed (Fig. 3).

3.1.1.3. *Toxicant dose* 771 µg/l Pb

Gill epithelium was destroyed in many areas, so that one might say that the epithelial arrangement was no more discernible. In these areas, the cell limits

disappeared or ctenoid epithelium had various heights and sometimes pseudostratification. The cell producing mucus had either spherical, pycnotic nuclei or they were adjacent and completely deformed; ovoid nuclei had a degraded chromatin. In the cytoplasm of confluated epithelial cells, a large vacuole was distinct. Connective tissue in gill axis was destroyed as well. The amoebocytes occurring in the connective tissue often pushed the basal pole of epithelial cells or even penetrated the epithelium. Generally, both connective cells and amoebocytes were completely deformed and their nuclei were pycnotic. Blood greatly dilated sinuses exhibited a destroyed epithelium (Fig.4).

3.1.2. Time of lead action 72 hours

3.1.2.1. *Toxicant dose of* 210 µg/l Pb

Ctenoids apparently maintained their shapes, although they exhibited a pronounced structural degradation. Many areas of the epithelium were pseudostratified; cell limits were no more discernible. Here and there spherical nuclei of the mucous cells, or elongated nuclei of cylindrical cells, which were pycnotic, occurred. Cell apical extremity was in general destroyed. Connective tissue in gill axis was degraded having both connective cells and destroyed amoebocytes. Blood sinuses with disrupted epithelium were often confluated (Fig. 5).

3.1.2.2. *Toxicant dose of* 289 µg/l Pb

Gill epithelium was completely destroyed; cell limits disappeared and epithelial cell nuclei, were generally no more discernible. Although, here and there, in the presumed epithelium, pycnotic nuclei traces were discernible. At the apical pole of cell traces, some cilia appeared as well. The connective tissue in ctenoid axis was completely destroyed and the blood sinuses were confluated (Fig. 6).

3.1.2.3. *Toxicant dose of* 771 µg/l Pb

Structural changes were similar to those generated by the previous dose, the epithelium having destroyed cells. Toward ctenoid base, in epithelium some nuclei were noticed, although cell limits were not discernible. Toward the apical end of gill lamella, some nuclei of unequal sizes and deformed contour could be noticed: many of them were pycnotic. The limits between cells were not discernible at this end either. In the connective tissue, the nuclei were pycnotic and blood sinuses had disrupted or greatly dilated walls.

3.1.3.Time of lead action 7 days

3.1.3.1. *Toxicant dose* 210 µg/l Pb

Only ctenoid contour was noticed, the epithelium being completely destroyed. Traces and elongated, pycnotic nuclei were noticed, especially at the

apical end of ctenoid. In degraded connective tissue, all the nuclei were destroyed. Degraded amoebocytes were discernible as well (Fig. 7).

3.1.3.2. *Toxicant dose* 289 µg/l Pb

Gill lamellae exhibited very pronounced structural changes, their contour hardly being noticed. Epithelial cells were completely destroyed, remaining traces of irregular shape and pycnotic nuclei. Thus, instead of cells, a mass of cytoplasm appeared with all kinds of cell remains. Connective tissue was completely destroyed; within it, here and there amoebocytes were noticed as well, which, somehow maintained their normal shapes, but blood sinuses were disrupted and fused (Fig. 8).

3.1.3.3. *Toxicant dose* 771 µg/l Pb

Gill degradation was very pronounced. Here and there traces of gill epithelim as well as scattered nuclei remained. Connective tissue was completely destroyed and therein, generally, no cell category could be discernible. Blood sinuses were destroyed (Fig. 9).

3.2. STRUCTURAL CHANGES AT INTERNAL GILL LEVEL

3.2.1.Time of lead action 24 hours

3.2.1.1. *Toxicant dose* 210 µg/l Pb

As in external gill case, at the level of internal gill we noticed that starting even with this dose the gill epithelium began to disorganize during minimal exposure time (24 hours). In this respect, the cells settled and the limits between them generally were lost and some nuclei became pycnotic. Cell basal pole was affected, where plasmalemma disrupted and thus connective tissue penetrated the cytoplasm of epithelial cells. Generally, connective tissue degraded connective cells having deformed and pycnotic nuclei. Collagen fibres were destroyed as well. In connective tissue and sinuses, in certain areas, agglomerations of black colour granules were noticed. Probably, they represent the toxic substance accumulated in a greater quantity in the respective portion. Many amoebocytes had pycnotic nuclei and they also exhibited black granulations in the cytoplasm. We think that these granulations probably represent cell lysosomes which accumulated the lead (Fig. 10).

3.2.1.2. *Toxicant dose* 289 µg/l Pb

Gill epithelium was strongly affected; in many areas it is completely degraded. Connective tissue was also degraded. Some black granules were found in gill axis (Fig. 11). The same granules representing the lead were present in amoebocytes as well. We think that may be the accumulation is less pronounced in the internal gill, the external one being the first barrier through which the pollutant passes and which is strongly destroyed.

3.2.1.3. Toxicant dose 771 µg/l Pb

At this dose, a very pronounced epithelium degradation was found. Thus, the cells were no more discernible; in many areas nuclei agglomerations were noticed and toxic granulations were accumulated within the epithelium. Connective tissue was also strongly degraded; in it, traces of various cell categories (connective ones and amoebocytes), as well as granule agglomerations (Fig.12) were noticed.

3.2.2. Time of lead action 72 hours

3.2.2.1. *Toxicant dose* 210 µg/l Pb

Gill epithelium remained with an apparent structural organization in some areas; in others, cell limits disappeared and nuclei became pycnotic. Cell basal pole was distrupted, connective tissue was infiltrated toward epithelium. In the very affected connective tissue, both cells and collagen fibres were destroyed. In blood sinuses, also degraded, accumulations of toxic substance were found (Fig. 13).

3.2.2.2. *Toxicant dose* 289 µg/l Pb

In the destroyed gill epithelium, cell limits were no more discernible. In the degraded connective tissue, black granulations were noticed. Destroyed blood sinuses confluated (Fig. 14).

3.2.2.3. *Toxicant dose* 771 µg/l Pb

Gill epithelium had cell limits disappeared and basal pole plasmalemma of some cells destroyed in such a way that the connective tissue as well as the existing granulations penetrated the epithelium. Toxic substance accumulation was also found in blood sinuses.

3.2.3. Time of lead action 7 days

3.2.3.1. *Toxicant dose* 210 µg/l Pb

Upon this dose action, but for a longer exposure time, only gill lamellae contour was maintained. Both apical and basal poles of epithelial cells were destroyed. Connective cells as well as amoebocytes invaded the gill epithelium. All the nuclei were destroyed. Deposits of toxic substance granules were more numerous in sinuses and the connective tissue (Fig. 15).

3.2.3.2. *Toxicant dose* 289 *and* 771 µg/l Pb

Structural degradation was more pronounced at these doses after 7 day – lead action, when only gill lamellae contour was discernible. In general, cells were destroyed and the remaining ones were crowded toward the apical pole of gill lamellae. Just in this portion, plasmalemmas were disrupted, so those nuclei appeared scattered. In gill lamella remainder, the epithelium was so destroyed that it was no more discernible. Connective tissue of gill axis was close to

disappearance and here and there isolated nuclei or deposits of toxic substance granules were noticed (Fig. 16).

4. DISCUSSION

Some metals influence on fresh or seawater mollusca was studied as we have mentioned in the introduction, by a number of authors. But, most of them considered the survival period, lethal doses, bioaccumulation, bioconversion [2, 3, 11]. In this respect, some of works present the results of studies concerning various relationships between two or several metals, the resultant being the synergy. Other studies outline the interaction leading to the decrease in some metals' accumulation (cadmium, copper), just at *Anodonta cygnaea* gill level [3].

As concerns the lead accumulation in *Mytilus edulis* gills, Marshall and Talbot found the presence of this metal in the form of extracellular crystalline deposits at basal lamina level, forming capillary walls of gill lamellae [6].

Our investigations outline the presence of some copper zinc and lead mineral deposits in the connective tissue of gill axis, as well as in amoebocytes in *Anodonta cygnaea* species [5, 9].

As concerns the structural changes at the level of various cells and tissues of the previously mentioned species were stressed in some works that show the action of copper, mercury, zinc [5, 8, 9].

The specialty literature outlines the fact that the response of organisms, exposed to the influence of various toxic substances, could be similar [7].

By our studies we found the similarity of a number of structural changes at the level of external and internal gills in *Anodonta cygnaea*, upon the action of several metals. Thus, we notice the degradation, even destruction of gill epithelium, connective tissue, amoebocyte, blood sinus alteration in this species specimens exposed to the influence of copper, zinc, mercury and lead existing in excess in water.

If certain structural changes caused by different metals are similar, the toxicant doses causing them vary. A comparative analysis concerning copper, zinc and lead action shows that the strongest action is that of lead, its toxic doses being much lower.

5. CONCLUSIONS

1. The lead (from lead acetate) at 210, 289 and 771 µg/l concentrations for a period ranging between 24 hours and 7 days caused significant structural changes in internal and external gills of *Anodonta cygnaea* molusc.

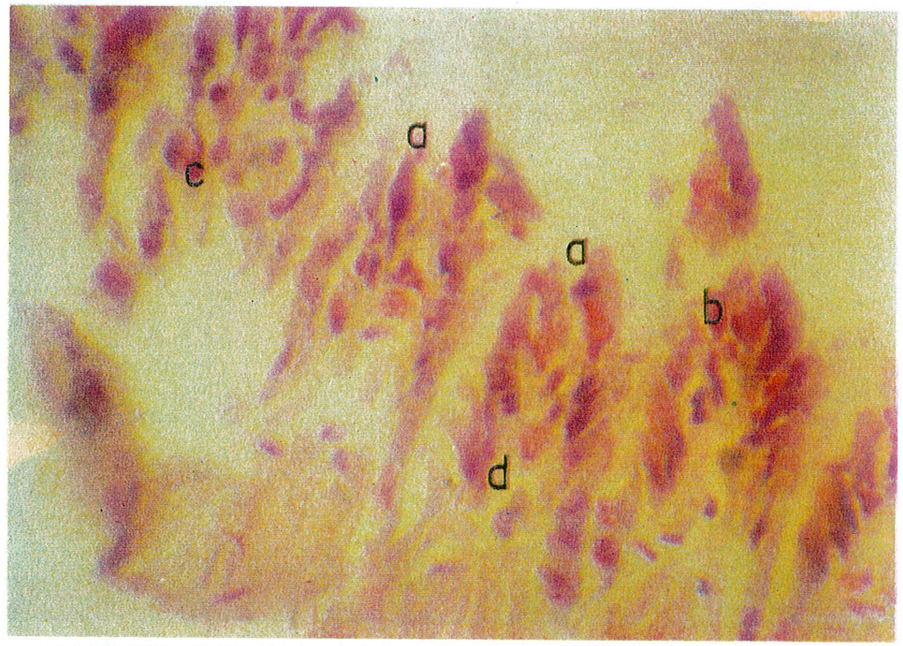


Fig. 1. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 24 hours: a = disorganized gill epithelium; b = pycnotic nuclei; c = amoebocytes; d = connective tissue (40 · 0.65).

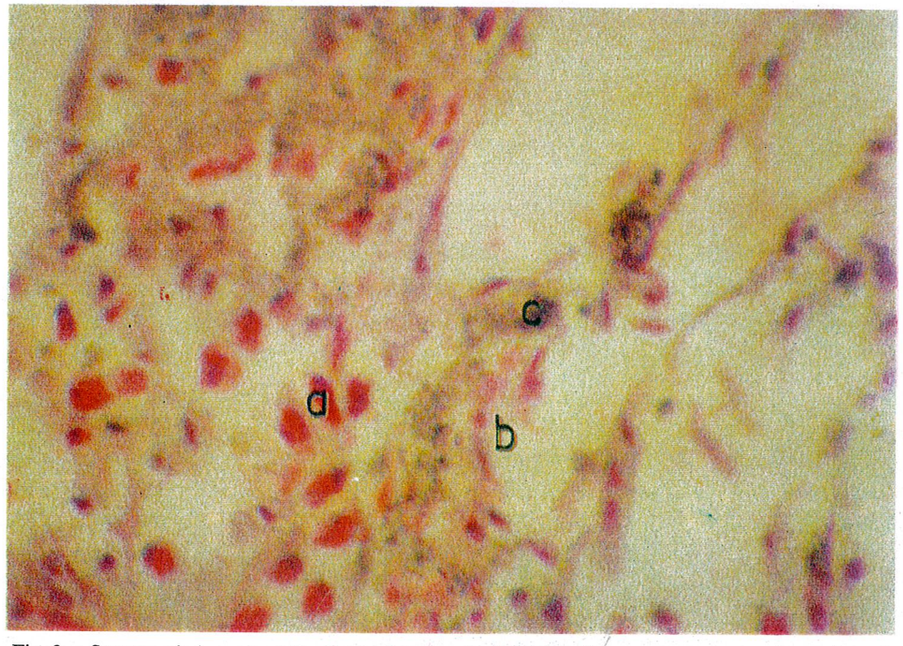


Fig. 2. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 24 hours: a = amoebocytes; b = blood sinuses; c = lead granules (40 · 0.65).

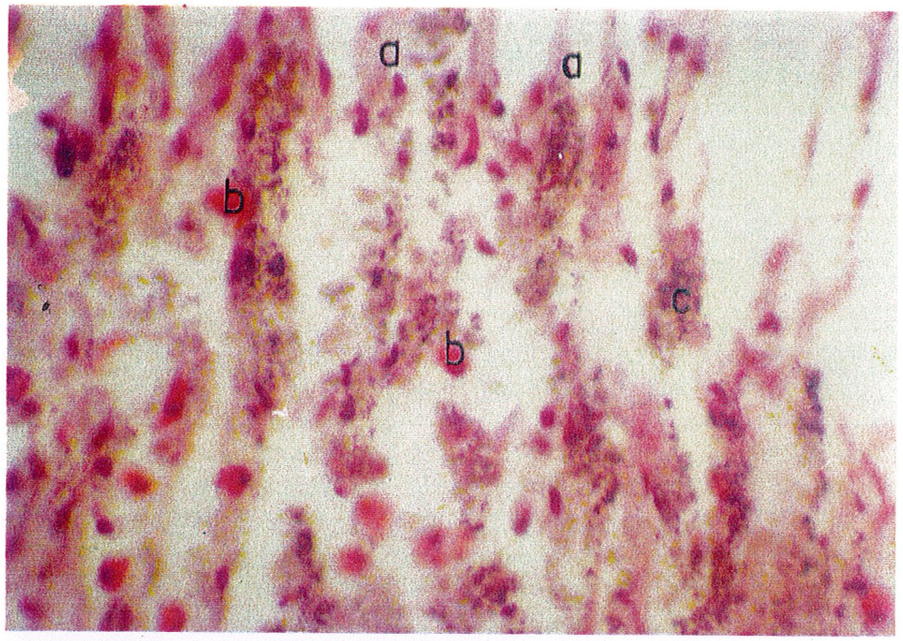


Fig. 3. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 µg/l Pb concentration, for 24 hours: a = disorganized gill epithelium; b = amoebocytes; c = granules deposits (40 · 0.65).

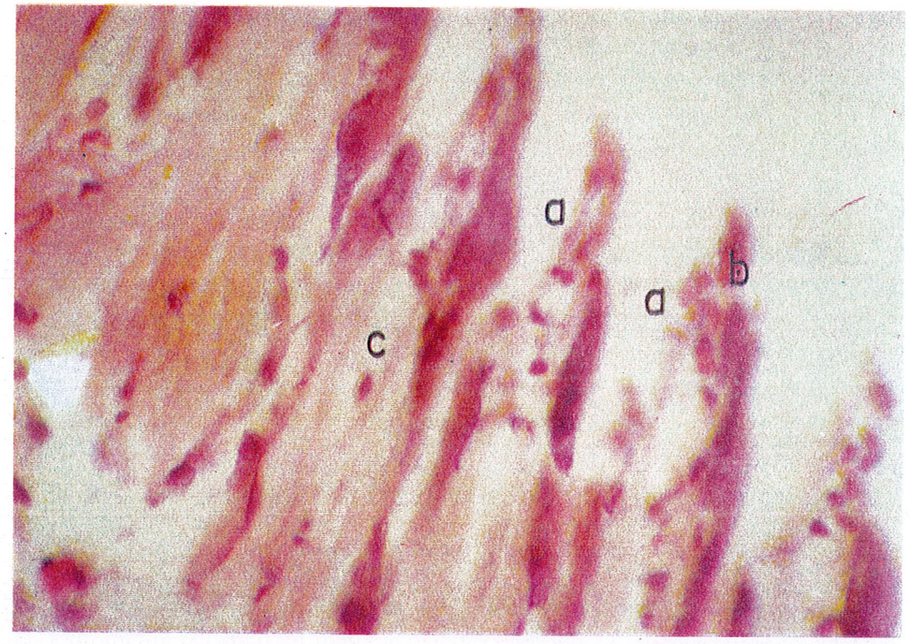


Fig. 4. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 771 µg/l Pb concentration, for 24 hours: a = completely destroyed gill epithelium; b = amoebocytes; c = degraded connective tissue (40 · 0.65).

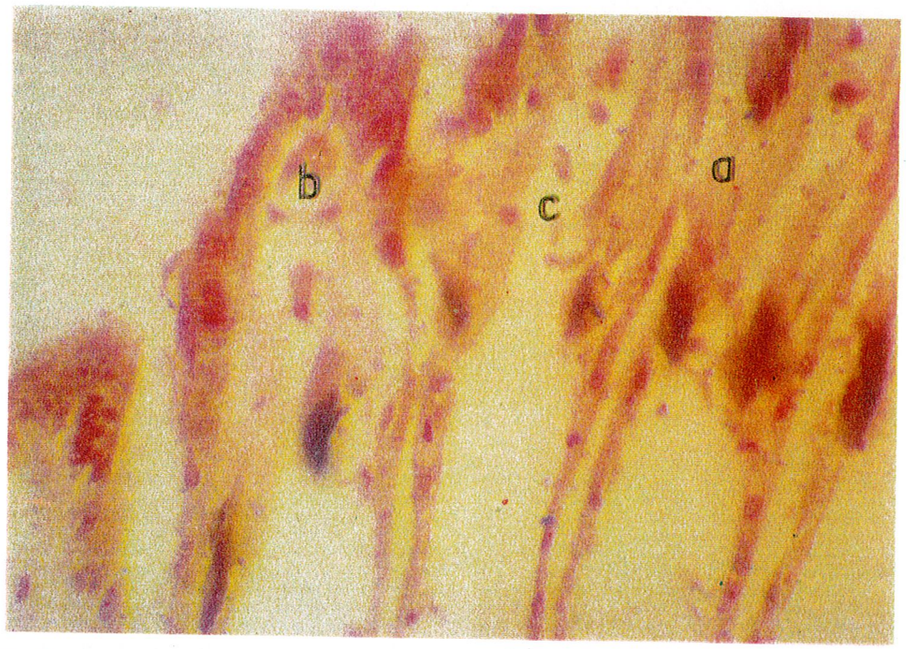


Fig. 5. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 72 hours: a = disorganized gill epithelium; b = amoebocytes; c = connective tissue (40 · 0.65).

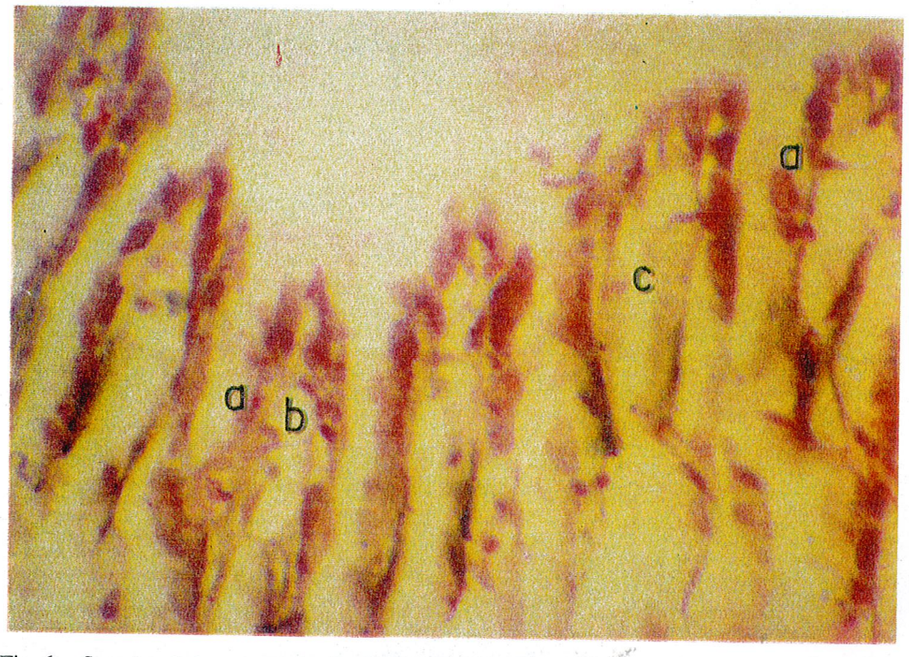


Fig. 6. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 µg/l Pb concentration, for 72 hours: a = destroyed gill epithelium; b = confluated blood sinuses; c = completely destroyed connective tissue (40 · 0.65).

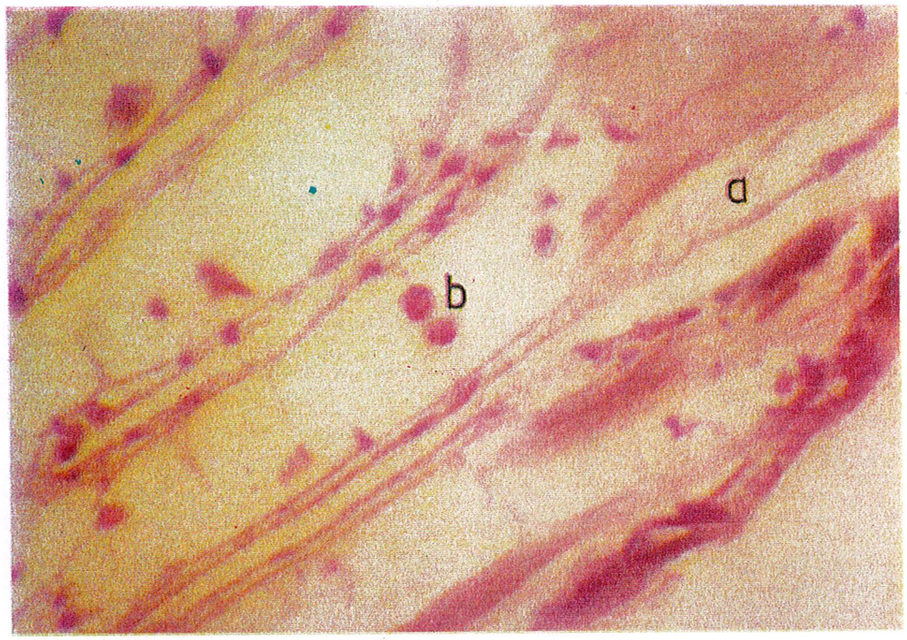


Fig. 7. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 7 days: a = disorganized gill epithelium; b = amoebocytes (40 · 0.65).

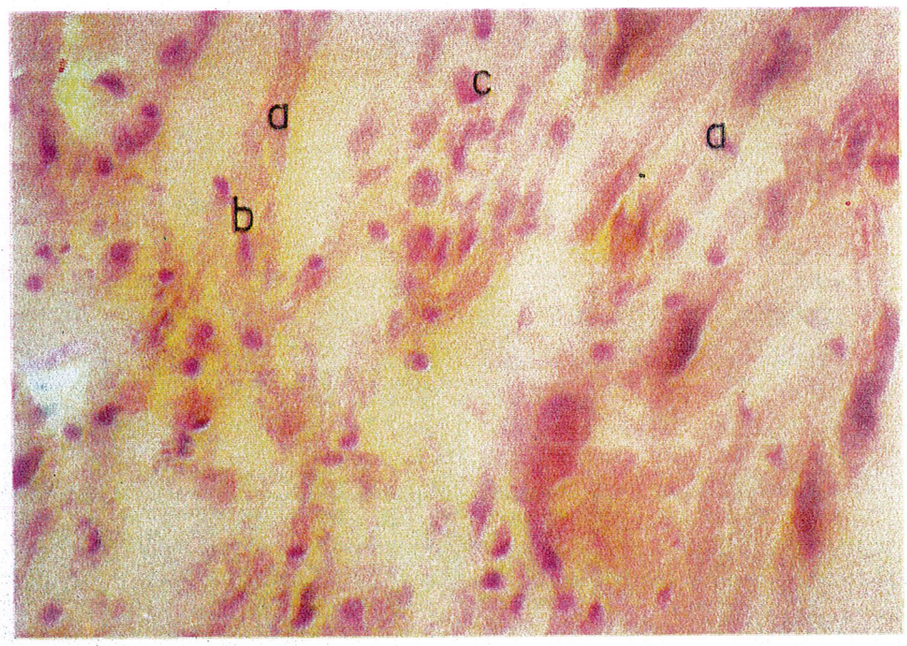


Fig. 8. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 µg/l Pb concentration, for 7 days: a = very degraded gill epithelium; b = nuclei of irregular shapes; c = amoebocytes (40 · 0.65).

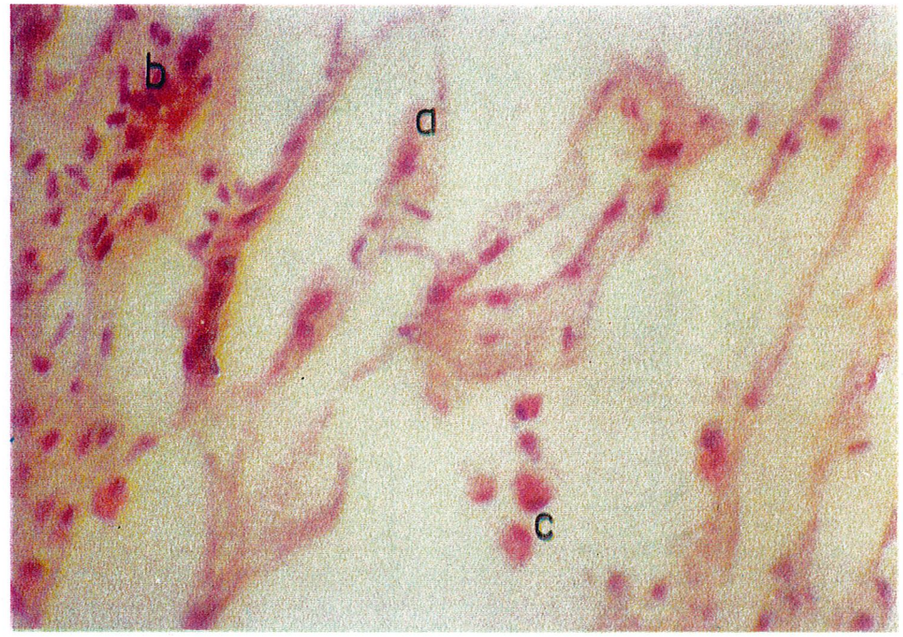


Fig. 9. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 771 µg/l Pb concentration, for 7 days: a = gill epithelium; of very pronounced degradation; b = scattered nuclei; c = amoebocytes (40 · 0.65).

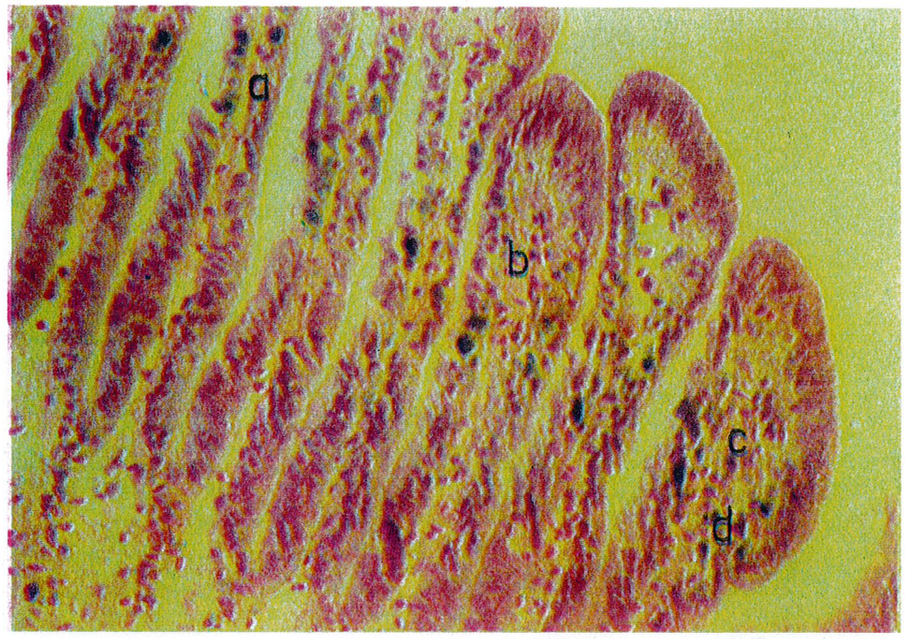


Fig. 10. – Structural changes at the level of internal gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 24 hours: a = settled epithelial cells with disrupted basal pole; b = degraded connective tissue; c = amoebocytes with granulations; d = granules' agglomerations (20 · 0.40).

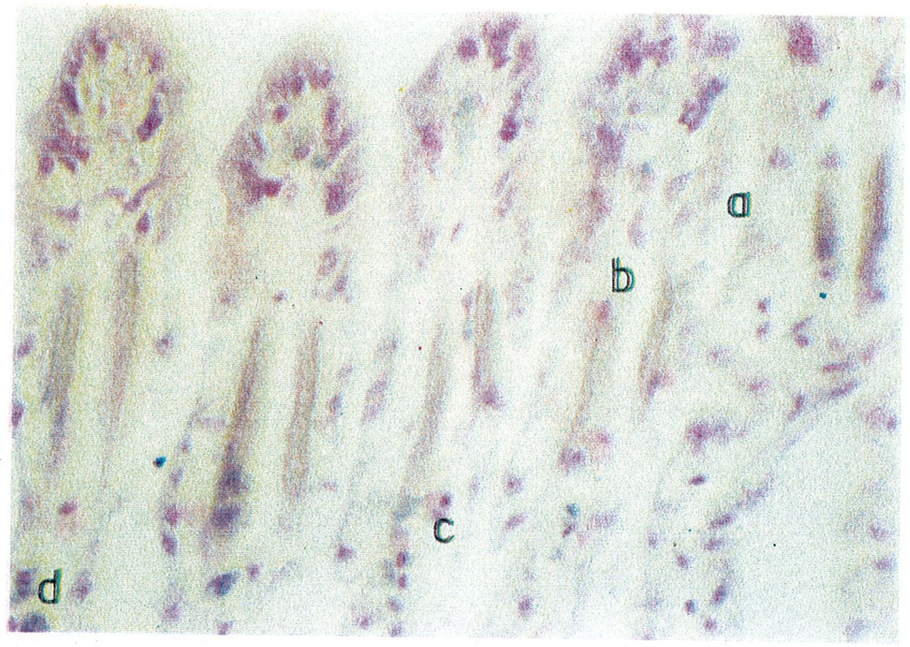


Fig. 11. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 µg/l Pb concentration, for 24 hours: a = disorganized gill epithelium; b = connective tissue; c = amoebocytes; d = lead granules (40 · 0.65).

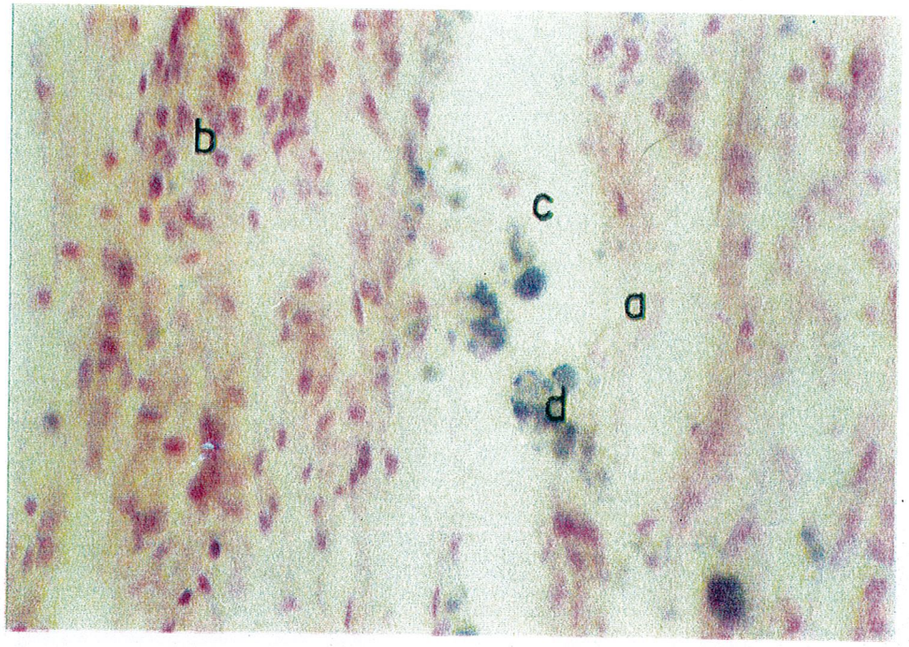


Fig. 12. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 771 µg/l Pb concentration, for 24 hours: a = strongly disorganized gill epithelium; b = nuclei agglomerations; c = degraded connective tissue; d = granules agglomerations (40 · 0.65).

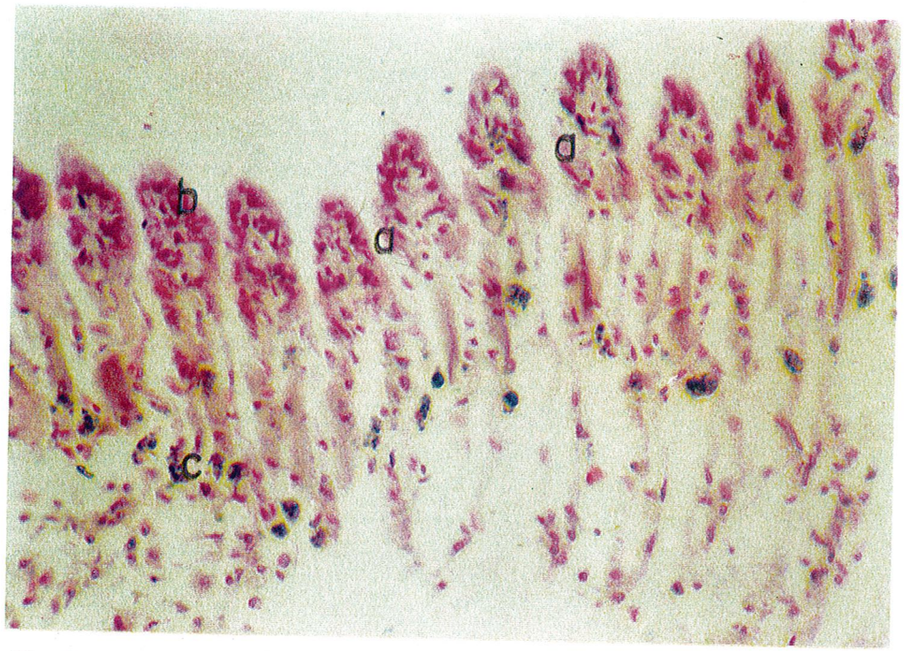


Fig. 13. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 72 hours: a = gill epithelium with an apparent organization; b = gill epithelium with disappeared cell limits; c = granules' agglomerations (20 · 0.40).

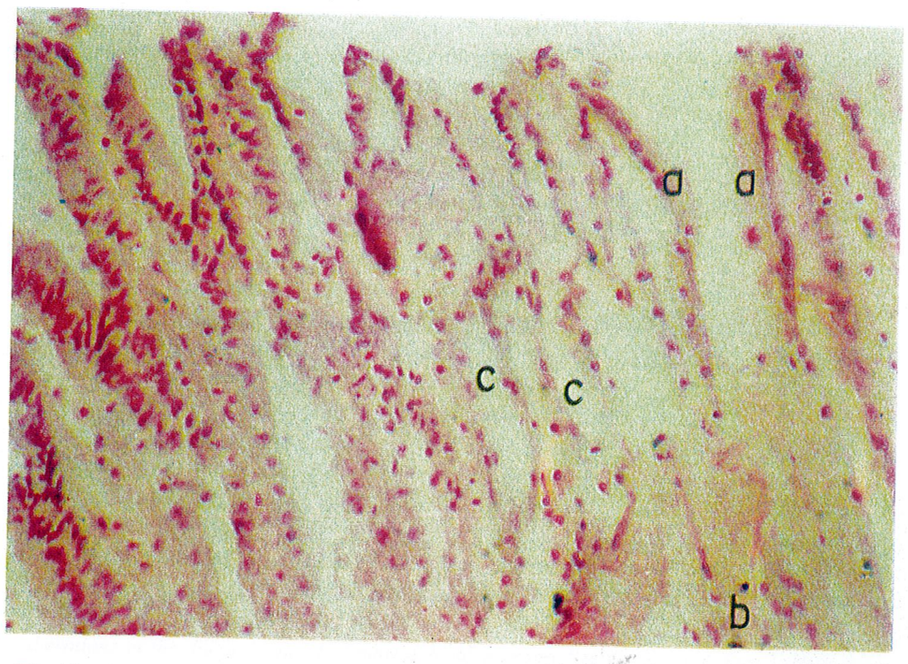


Fig. 14. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 µg/l Pb concentration, for 72 hours: a = disorganized gill epithelium; b = lead granules; c = confluated blood sinuses (20 · 0.40).

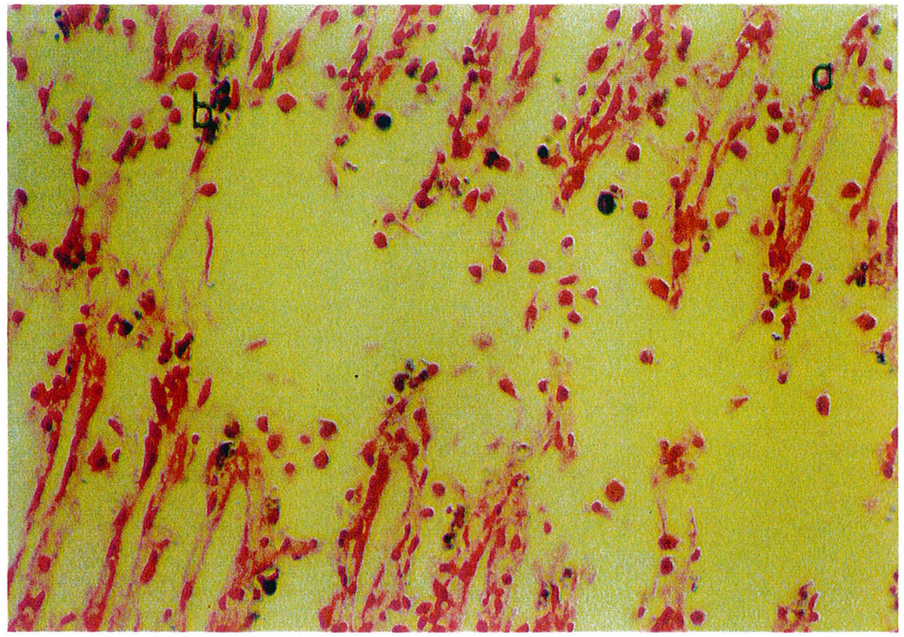


Fig. 15. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 210 µg/l Pb concentration, for 7 days: a = gill lamella contour; b = lead granules; (20 · 0.40).

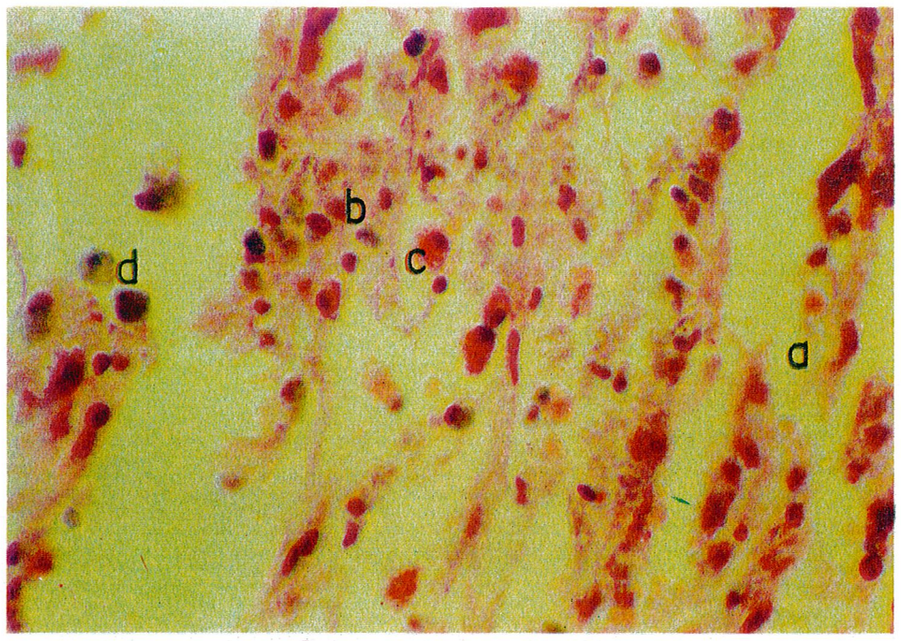


Fig. 16. – Structural changes at the level of external gill in *Anodonta cygnaea,* upon the action of 289 and 771 µg/l Pb concentration, for 7 days: a = very strongly disorganized gill epithelium; b = scattered nuclei; c = amoebocytes; d = lead granules (40 · 0.65).

2. Major structural changes both in external and internal gills occurred even upon the action of the lowest lead concentration tested by us (210 µg/l) and during the shortest exposure period (24 hours) of organisms.

3. Major structural changes at the gill level found by our researches are: a) gill epithelium degradation by cell limit disappearance, cytoplasm scattering, nuclei pycnosis; b) degradation of connective tissue, amoebocytes, blood, sinuses; c) toxicant granules' accumulation at the level of different cell categories.

4. Although cell and tissue changes generated by lead doses used by us were not lethal for the analysed *Anodonta cygnaea* specimens, for 7 days, but they were major and irreversible; they became lethal for a longer period.

5. Structural changes at the gill level were generally directly proportional to lead concentration and time of organism exposure to this metal action.

REFERENCES

1. Engel D.W., Fowler B. A., *Copper and cadmium induced changes in the metabolism and structure of molluscan gill tissue,* Marine Pollution: Functional responses, Acad. Press, Inc., London, 239–256 (1979).
2. George S. G.,*Correlation of metal acumulation in mussels with the mechanisms of uptake, metabolism and detoxification,* Thalassia Jugoslavica **16**, 347–365 (1980).
3. Holwerda D. A., *Cadmium kinetics in freshwater clams. V. Cadmium-Copper interaction in metal accumulation by Anodonta cygnaea and characterization of the metal-binding protein,* Arch. Environ. Contam. Toxicol., **21**, *3*, 432–437 (1991).
4. Linnik P. N., Nabiwaner J. B., Osadtschaja N. N., *Konzentrationen und Migrationsformen der Schwermetalle im Donauwasser,* in *Ergebnisse der Internationalen Donauexpedition 1988*, 77–85, Wien (1990).
5. Manolache Viorica, Popescu-Marinescu Virginia, Năstăsescu Maria, Marinescu Carmen, *Structural changes induced by copper intoxication in Anodonta cygnaea L. (Lamellibranchiata) gills and hepatopancreas,* Rev. roum. biol.,biol. anim., **42**, *1*, 41–48, Bucarest (1997).
6. Marshall A. I., Talbot V., *Accumulation of cadmium and lead in the gills of Mytilus edulis: X - ray microanalysis and chemical analysis,* Chem. Biol. Interact. **27**, *1*, 111–123 (1979).
7. Mălăcea I., *Biologia apelor impurificate*, Ed. Acad. RSR., 1969, 142–181.
8. Năstăsescu Maria, Manolache Viorica, Zărnescu Otilia, Tesio C., *The action of mercury on the digestive tract at Anodonta cygnaea piscinalis (Lamellibranchiata, Bivalva),* Rev. roum. biol., **40**, *1*, 33–37, Bucarest (1995).
9. Năstăsescu Maria, Popescu-Marinescu Virginia, Manolache Viorica, Marinescu Carmen, *Acţiunea zincului asupra mantalei, branhiilor şi hepatopancreasului de la Anodonta cygnaea (Mollusca-Lamellibranchiata),* St. cerc. biol., Seria biol. anim., **49**, *2*, 147–155, Bucureşti (1997).
10. Sarasquette E. C., Gonzales de Canales M. L., Gimeno S., *Comparative histopathological alterations in the digestive gland of marine bivalve exposed to Cu and Cd,* Eur. J. Histochem., **36**, *2*, 223–232 (1992).

11. Wachs B., *Schwermetallgehalt des Zoobenthos der Donau*, in *Limnologische Berichte Donau*, I, 30.Arbeitstagung der IAD, ZOUZ-Schweiz 1994, Wissenschaftliche Kurzreferate, 300–309 Düßendorf (1994).

Received June 28, 1999.

**National Institute of Research and Development for Biological Sciences, Spl.Independenţei 296, Bucharest*

*** Faculty of Biology, Spl. Independenţei 93–95. Bucharest*

STRUCTURAL CHANGES CAUSED BY LEAD ACTION AT THE LEVEL OF *ANODONTA CYGNAEA* L. (MOLLUSCA, LAMELLIBRANCHIATA) MANTLE

MARIA NĂSTĂSESCU*, VIORICA MANOLACHE*, VIRGINIA POPESCU-MARINESCU**, DANIELA TEODORESCU**, ELENA NEAGU**

Investigations concerning the influence of lead (from lead acetate), under experimental conditions (static tests), at initial concentrations of 210; 289 and 771 µg/l Pb at various times, ranging from 24 hours to 7 days of organism exposure to toxicant action revealed a number of structural changes at the level of *Anodonta cygnaea* mantle. In this respect, the lead action upon mantle structure was materialized by significant changes beginning even with the smallest dose used (210 µg/l Pb) during the shortest time of organism exposure (24 hours). The mantle structure disorganization was more pronounced as the lead doses and time of exposure increased. Very pronounced degradation of all the mantle cell and tissue structures was however found at 771 µg/l lead concentration for 7 day – intoxication. In this case, the mantle epithelium was disintegrated, the cells were completely destroyed, nuclei were deformed and pycnotic.

In some areas, the epithelium was even transformed into a granular mass. Connective tissue was degraded too, and cell nuclei appeared pycnotic in it. Blood sinuses had disrupted walls. Muscle fibre myofibrils were also destroyed.

1. INTRODUCTION

Statistical data show that during the last decade, due to intensification of industrial activity and used water discharges into surface natural recipients, the amounts of heavy metals increased very much in different aquatic basins.

One of these heavy metals of high toxicity for organisms, when certain limits are exceeded, is lead, a particularly dangerous pollutant, since it has an extremely low rate of biological degradation, it does not precipitate in the sediment at the bottom of the aquatic basin, but it accumulates in the basin.

As concerns the resistance of various groups of aquatic organisms to lead action, a certain gradation was noticed. Thus, the sensitivity to this metal ion decreases in the following order: fish, mollusca, worms (oligochaetes, hirudins), trichopteres, etc. Hence, the filtrators, lamellibranchiated mollusca, respectively, are the most sensitive invertebrates to lead influence. Filtrators also intensely accumulate the pollutant from water in their bodies.

REV. ROUM. BIOL. – BIOL. ANIM., TOME 44, N° 2, P. 189–195, BUCAREST, 1999

In this respect, the researches performed by Wachs found in a kilogram of wet substance of *Lithoglyphus naticoides*, originating from different portions of the Danube, variable amounts of lead ranging from 11 mg (at 790 km – Vidin) to 2.5 mg (at 93 km – Ismail) [12]. For *Unio pictorum* lamellibranchiatum (also from 790 km) the same author mentioned 2.2 mg Pb/kg wet substance of mollusc.

But the highest accumulation of lead was found in the sediment of the Danube bottom, where in 1990 the amounts varied between 42.5 mg/kg dry sediment (at km 2162.820 – Aschach) and 69.3 mg/kg (at km 1949.330 – Greifenstein) [13]. But in the lower zones of the river, in 1988 the sediment lead values were sometimes much higher, namely 177.6 µg/g (at km 790); but toward the river mouth they decreased to 82.8 µg/g (at km 131.5 – Reni) or even to 62.8 µg/g (at km 93) [2]. According to other author at the same km 93, the amount of lead from the sediment was of 20–41 mg/kg only[12].

But, in the Danube, in 1991 the lead was present under < 0.002 – 0.003 mg/l in higher zones (at km 2203.800), but pollutant quantity increased toward the river mouth, where it reached 0.009 – 0.039 mg/l (at km 131.5) [13]. A value close to the last figure, i.e. 0.030 mg/l was mentioned in the Danube river of Soviet, Romanian, Bulgarian and Yugoslavian sectors, for 1988 [5].

Taking into account all these data and considering the fact that the researches carried out by one of this work's authors (V. Popescu – Marinescu) in 1995–1996 have revealed a higher mortality of *Viviparus acerosus* and *Unio tumidus* species of the Danube, we directed our researches to discover the changes occurring upon lead action at structural level in *Anodonta cygnaea* lamellibranchiatum.

We outline the fact that if this species is present in the Danube in small quantities, it plays an important role in the economics of many internal aquatic basins. On the other hand, certain lamellibranchiata, among which the analysed species is present, are used in the food industry.

2. MATERIALS AND METHODS

In order to show as accurately as possible the structural changes at mantle level in *Anodonta cygnaea* caused by lead action (from lead acetate), at different concentrations and for various times, first we performed a number of experiments. In this respect, batches of tens of adult specimens of this lamellibranchiatum were exposed, through static trials, to the influence of lead from solutions at concentrations of 210, 289 and 771 µg/l. Then, certain specimens among those intoxicated in this way were killed and dissected at different periods of time (of maintaining in noxious solution), namely for 24 hours, 72 hours and 7 days. Portions of mantle were immediately fixed in Bouin; then, they were processed and analysed by classic methods used by light microscopy.

3. RESULTS

3.1. TIME OF ORGANISM EXPOSURE TO LEAD ACTION 24 HOURS

3.1.1. *Toxicant dose* 210 µg/l Pb

Lead action upon *Anodonta cygnaea* mantle structure was shown by significant changes beginning even with the smallest dose used and for the shortest time of organism exposure. Under such conditions, our observations showed that the unistratified epithelium of mantle was affected toward both the two mantle poles and the middle of it. In these areas, the intoxicated animal cells were low, settled compared to those belonging to control. But, prismatic cell cilia were distinct, as well as a thin layer of mucus at epithelium surface. In the connective tissue underlying the epithelium, some deformed and pycnotic nuclei were seen. Also, muscle fibres, orientated to all the directions, had pycnotic nuclei in many cases (Fig.1).

3.1.2. *Toxicant dose* 289 µg/l Pb

As it happens more seldom, (when higher concentrations of a substance or element prove to be less toxic than smaller ones), in some specimens introduced in 289 µg/l Pb dose, the mantle epithelium in some zones was less modified than at 210 µg/l Pb. Thus, in these portions, epithelium appeared as being intact, both mucous cells and prismatic ciliated cells were well discernible. In very few zones the epithelium was much changed, and the cells were destroyed there. But connective tissue was greatly affected, especially connective cell nuclei were deformed and pycnotic (Fig. 2).

3.1.3. *Toxicant dose* 771 µg/l Pb

Most of mantle epithelium was affected. Thus, mucous or prismatic cells were fused, cell limits disappearing and nuclei were degraded. In the connective tissue both connective cells and blood sinuses were affected, exhibiting disrupted walls (Fig.3).

3.2. TIME OF ORGANISM EXPOSURE TO LEAD ACTION 72 HOURS

3.2.1. *Toxicant dose* 210 µg/l Pb

On this more prolonged exposure, in the epithelium, prismatic cells of the central zone were settled, sometimes they were even completely destroyed; cells of the peripheral zone were smaller. Connective tissue was, generally, modified as well (Fig.4).

3.2.2. *Toxicant dose* 289 µg/l Pb

As in the case of animal exposure for 24 hours at this lead concentration, although the period of exposure was extended to 72 hours, in some portions, the mantle epithelium had an almost normal appearance. Yet, in the central zone it was pluristratified and nuclei were pycnotic. The connective tissue underwent great changes as well (Fig. 5). In this respect, there were noticed disrupted or dilated blood sinuses and in the cytoplasm of some amoebocytes black granules which, in our opinion, represent a toxic substance.

3.2.3. *Toxicant dose* – 771 µg/l Pb

In a great part, the epithelium was destroyed, from it remaining but a few deformed, pycnotic nuclei and cytoplasm debris. In very few zones, a certain integrity of the epithelium was maintained. The connective tissue was affected both at the level of connective cell nuclei which appeared pycnotic and the blood sinuses which were disrupted. Apparently, muscle myofibrils were not affected.

3.3. TIME OF ORGANISM EXPOSURE TO LEAD ACTION 7 DAYS

3.3.1. *Toxicant dose* 210 µg/l Pb

During a prolonged time, but at the smallest concentration of lead in the solution in which mollusca were maintained, the mantle epithelium in some areas was, generally, little affected. Yet, portions of it appeared degraded, the cells being completely destroyed and nuclei pycnotic. Also, the connective tissue had destroyed cells, with degraded nuclei and blood sinuses had disrupted walls or they confluated (Fig. 6).

3.3.2. *Toxicant dose* 289 µg/l Pb

At this dose, the epithelium was degraded enough. In this respect, in some zones, both mucous cells and ciliated, prismatic cells appeared destroyed; in other portions, the cells were greatly flattened. The subjacent tissue pushed the basal pole of epithelial cells, so that a normal epithelial arrangement was no more apparent. Connective tissue was greatly affected, cell nuclei becoming pycnotic. Also, muscle fiber myofibrils were destroyed (Fig. 7).

3.3.3. *Toxicant dose* 771 µg/l Pb

A very pronounced degradation of all the cell and tissue structures in *Anodonta cygnaea* mantle was found at 771 µg/l Pb concentration for 7 days intoxication. In this case the mantle epithelium was disintegrated, the cells were completely destroyed, nuclei were deformed and pycnotic. In some areas, the

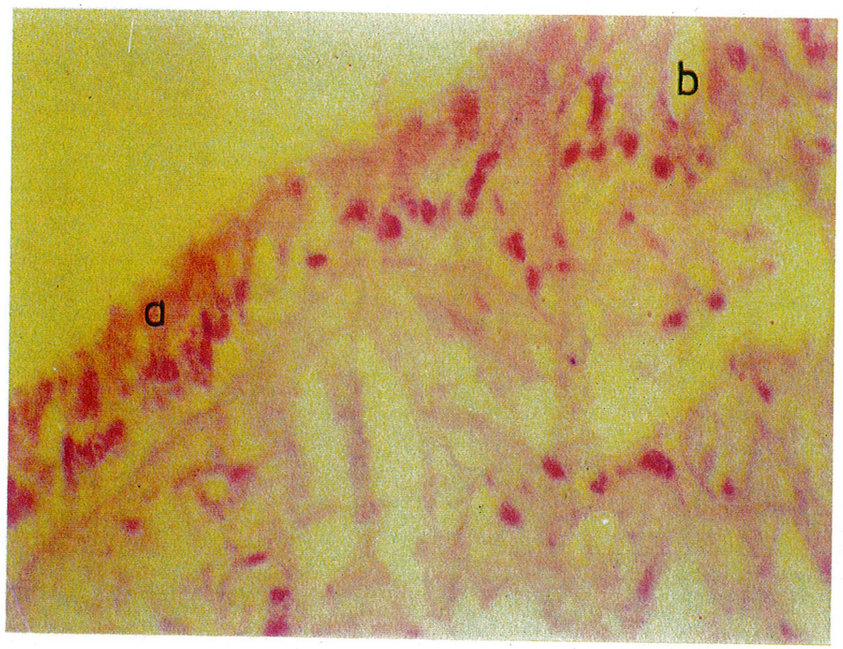


Fig. 1. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 210 µg/l Pb concentration, for 24 hours: a = prismatic cells; b = mucous cells (40 · 0.65).

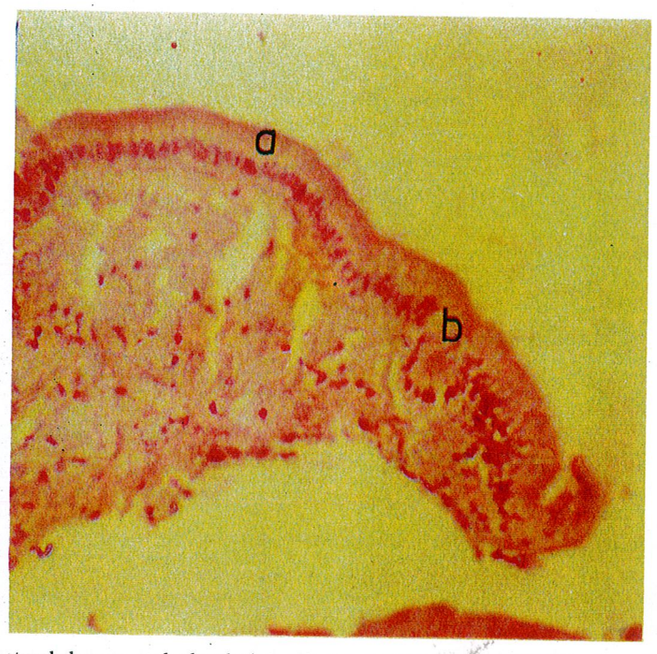


Fig. 2. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 289 µg/l Pb concentration, for 24 hours: a = prismatic cells; b = mucous cells (20 · 0.40).

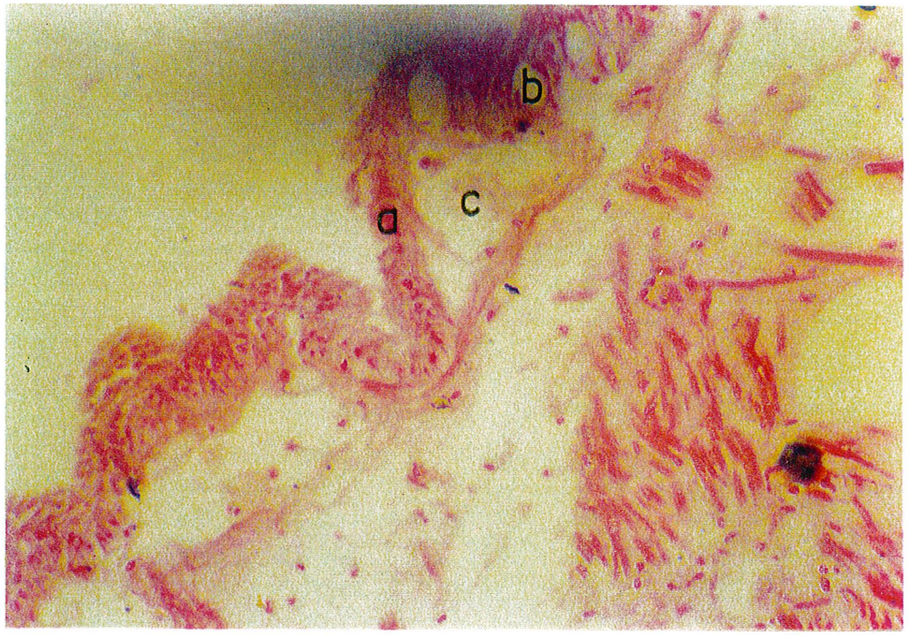


Fig. 3. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 771 µg/l Pb concentration, for 24 hours: a = prismatic cells; b = mucous cells; c = connective tissue (20 · 0.40).

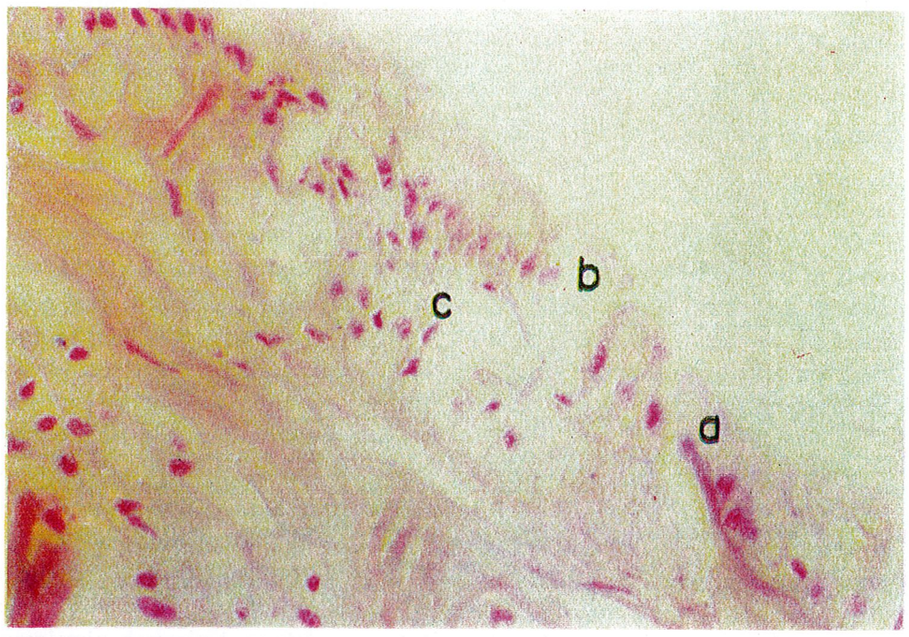


Fig. 4. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 210 µg/l Pb concentration, for 72 hours: a = settled cells; b = destroyed cells; c = connective tissue (40 · 0.65).

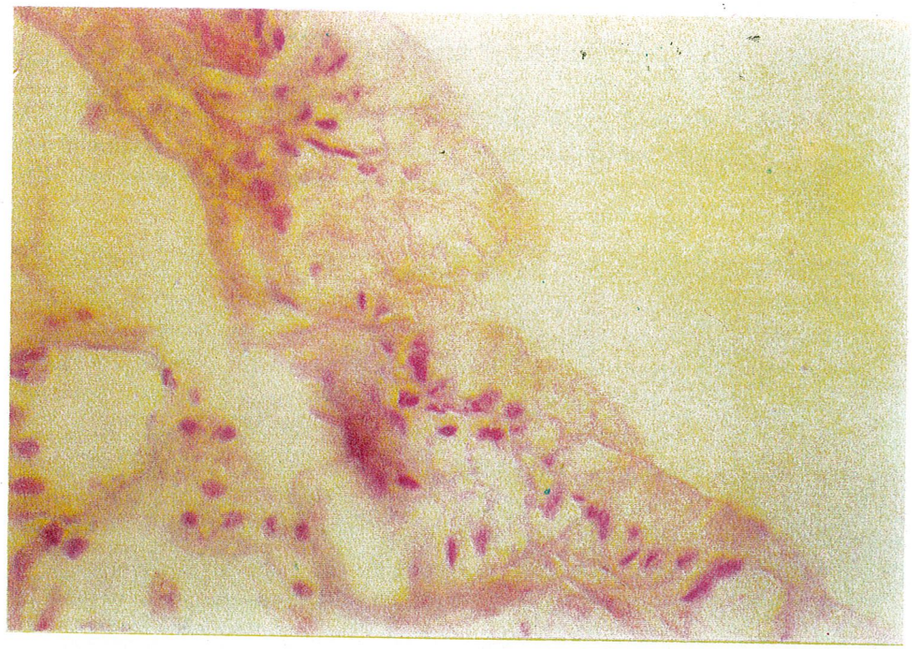


Fig. 5. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 289 µg/l Pb concentration, for 72 hours (40 · 0.65).

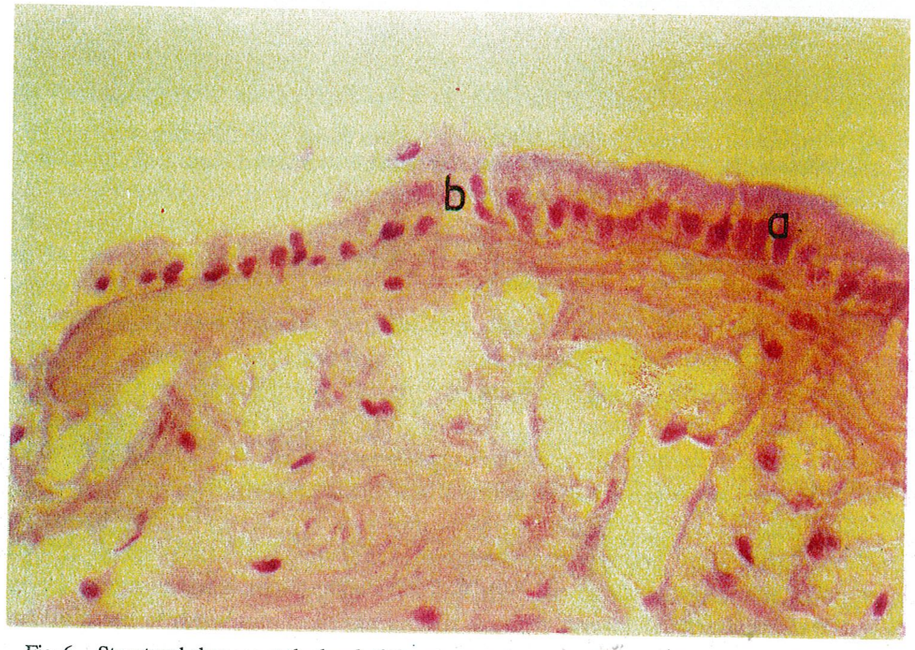


Fig. 6. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 210 µg/l Pb concentration, for 7 days: a = less affected epithelium; b = degraded epithelium (40 · 0.65).

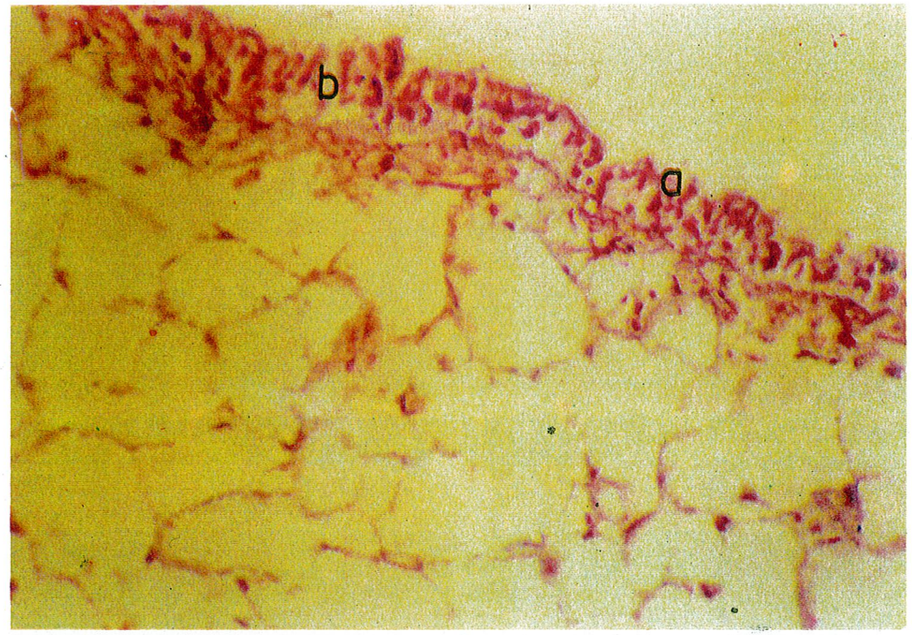


Fig. 7. – Structural changes at the level of *Anodonta cygnaea* mantle upon the action of 289 µg/l Pb concentration, for 7 days: a = degraded prismatic cells; b = degraded mucous cells (20 · 0.40).

epithelium was even transformed into a granular mass. Also, the connective tissue was degraded, its cell nuclei appearing pycnotic. Blood sinuses had disrupted walls. Muscle fibre myofibrils were destroyed too.

4. DISCUSSION

Studies concerning the influence of heavy metals on mantle in invertebrates are not numerous. Researchers as Andersen and Baatrup, Engel and Fowler, Năstăsescu et al., Popescu-Marinescu et al., Sunilla, describe nevertheless a number of changes generated mainly by mercury, copper, cadmium, iron, zinc and ammonium action [1, 3, 5, 7, 8, 11]. In these works, the authors mention the fact that at the mantle level of different mollusca species, a destruction occurred, both of ciliated cells and the mucous cells in the epithelium; also, cell limits disappeared and pycnotic nuclei appeared. At the same time, a destruction of the connective tissue from under the epithelium, of connective cells respectively, of amoebocytes in this tissue, as well as of venous sinuses occurred.

The same kind of changes were also noticed by us in *Anodonta cygnaea* mantle under the influence of lead from lead acetate.

The fact that various heavy metals cause similar changes in mollusca mantle is not a unusual phenomenon in animal world. A number of specialty works, particularly concerning the fish, mention such situations.

But it is noteworthy the fact that similar changes generated by different noxious substances are caused by totally different toxicant concentrations and times. Thus, the researches of our team show that changes consisting of the destruction of ciliated cells' and mucous cells' limits in the mantle epithelium, as well as connective cells, amoebocytes changes and blood sinuses in the connective tissue confluation, all of them occurred upon the influence of 0.3 mg/l Zn dose for 96 hours , for NH_4Cl at 15 mg/l for 24 hours and for lead at 771 µg/l for 24 hours [7, 8].

We must also outline the fact that the action of the same substance is expressed generally depending on its concentration and the time of animal exposure to toxicant influence. But the so-called "paradoxal effects" are known, as Schatz 1964 shows (mentioned by I. Mălăcea) [6], when small concentrations of some substances can be more deleterious than higher concentrations. The explanation varies from one case to another, but in some cases the way of action is unknown.

In this respect, by the present researches, we show that both for 24 hours and for 72 hours animals maintenance at 289 µg/l Pb concentration, the changes in certain areas of *Anodonta cygnaea* mantle are less pronounced than at 210 µg/l Pb concentration, for the same times of course.

On the other hand, a substance concentration which can cause chronic or acute intoxications depends on a) the velocity at which this substance can be converted, during metabolic processes, into nontoxic products or b) nondegradable accumulations can intervene in different vital organs of animals. In the case of lead, strictly speaking, a biodegradation does not occur in mollusca bodies, since this metal has an extremely low biological degradation rate.

But some authors reported in Gasteropod and Crustacean hepatopancreas the presence of some granules surrounded by membranes [4,9,10]. These granules include metals in the form of intracellular mineral deposits, thus contributing to the disintoxication of hepatopancreas cell cytoplasm.

We also found such agglomerations of metals in the form of granules too, in amoebocyte cytoplasm existing in the connective tissue of mantle and in the gills of *Anodonta cygnaea* specimens subjected to copper and lead influence.

Considering this evidence, we think that *Anodonta cygnaea* specimens of our experiments, although they did not die even at the highest dose of lead, 771 µg/l respectively for 7 days, yet due to major and irreversible changes occurring not only at the mantle level, but also at the level of other vital organs, they cannot survive for a longer period.

5. CONCLUSIONS

1. The lead action upon the mantle structure resulted in important changes beginning even at the concentration of 210 µg/l and for the shortest time of organism exposure, 24 hours.

2. Disorganization of mantle structure was generally more pronounced as lead concentrations in solution and time of exposure increased.

3. A very pronounced degradation of all the cellular and tissue structures of the mantle was seen at lead concentration of 771 µg/l, for 7 days.

4. Connective tissue exhibits a high sensitivity even at small doses and for a short time of exposure.

REFERENCES

1. Andersen J. T., Baatrup E., *Ultrastructural localization of mercury accumulations in the gills, hepatopancreas, midgut and antennal glands of the brown shrimps Crangon crangon,* Aquatic Toxicology, **13**, 309–324 (1988).
2. Belokonj W. N., *Schwermetalle in den Bodensediment der Donau*, in *Ergebnisse der Internationalen Donauexpedition, 1988*, 43–56, Wien (1990).
3. Engel D. W., Fowler B. A., *Copper and cadmium induced changes in the metabolism and structure of molluscan gill tissue,* Marine Pollution: Functional responses, Acad. Press. Inc., London, 239–256 (1979).

4. Hopkin P.S., Nott S. A., *Some observation on concentrically structural intracellular granules in the hepatopancreas of the shore crab Carcinus moenas L.*, J. mar. biol., ASS. U. K., **59**, 867–877 (1979).
5. Linnik P. N., Nabiwaner J. B., Osadtschaja N. N., *Konzentrationen und Migrationsformen der Schwermetalle im Donauwasser*, in *Ergebnisse der Internationalen Donauexpedition 1988*, 77–85, Wien (1990).
6. Mălăcea I., *Biologia apelor impurificate*, Ed. Acad., R.S.R., 149–181, 1969.
7. Năstăsescu Maria, Popescu-Marinescu Virginia, Manolache Viorica, Marinescu Carmen, *Acţiunea zincului asupra mantalei, branhiilor şi hepatopancreasului de la Anodonta cygnaea (Mollusca-Lamellibranchiata)*, St. cerc. biol., Seria biol. anim., **49**, 2, 147–155, Bucureşti (1997).
8. Popescu-Marinescu Virginia, Manolache Viorica, Năstăsescu Maria, Marinescu Carmen, Teodorescu Daniela, *Structural changes caused by ammonium chloride at the level of mantle and gills in Anodonta cygnaea L. (Mollusca, Lamellibranchiata)*, Rom. J. Biol. Sci., *1-2*, **II**, 89–101, Bucharest (1998).
9. Simina T., Boss J. L., *Structure and function of the calcium cells of the fresh water pulmonate snail Lymnaea stagnalis*, Netherlands J. of Zool., **27**, 195–208 (1977).
10. Simkins K., *Biomineralization and detoxification*, Calcified tissue research, **24**, 199–200 (1977).
11. Sunila I., *Copper and cadmium induced histological changes in the mantle of Mytilus edilis L. (Bivalvia)*, Limnologica, **15**, 523–527, Berlin (1984).
12. Wachs B., *Schwermetallgehalt des Zoobenthos der Donau, Schwermetallgehalt von Sedimenten aus der Donau*, in *Limnologische Berichte Donau, 1994*, I, 30. Arbeitstagung der IAD, ZOUZ-Schweiz 1994, Wissenschaftliche Kurzreferate, 300–309; 310–314, Düßendorf(1994).
13. Weber E., *Spezifische Schadstoffe. Schwermetalle im Wasser und Sediment*, in *Die Wasserbeschaffenheit der Donau von Passau bis zu ihrer Mündung, Ergebnisse der Donau – Forschung*, **2**, 245–253, Wien (1993).

Received June 28, 1999.

* *Faculty of Biology, Splaiul Independenţei 93–95, Bucharest*

** *National Institute of Research and Development for Biological Sciences, Spl. Independenţei 296, Bucharest*

BACTERIAL DENITRIFICATION OF MUNICIPAL WASTEWATER AT BUCHAREST-GLINA PURIFICATION PLANT

CONSTANTIN DELCEA

This paper concerns the bioremediation of the municipal wastewater. In this paper a microbiological analysis of the main physiological groups of bacteria both in the influent and effluent of the Bucharest-Glina purification plant has been done. It has been noticed that the most bacteria types have their cellular density about one hundred higher in the influent than in the effluent of the plant. The isolated bacteria have been noticed that bacteria provided from the influent belonged to 14 different bacterial genera.

Bacteria coming from the effluent belong to only 8 different bacterial genera. A program of "screening" has been made, due to which, 18 bacterial strains have been selected with a very high denitrification activity. The selected bacteria belong to *Pseudomonas, Micrococcus, Bacillus, Flavobacterium* genera and to an unidentified Gram-negative bacterial strain.

1. INTRODUCTION

From the quantitative point of view, the organic substances are the most important polluting factors on our planet. The removal of these polluting factors consists in their mineralisation to CO_2, H_2O, NH_4, and some low quantity of H_2S and H_2. Ammonium is oxidized into nitrate, a chemical stable substance from the chemical point of view and high level of oxidation. The removal of nitrate from wastewater is, usually, carried out by the denitrification process. The denitrification is a microbiologic process and it is exclusively realized by bacteria. It consists in disassimilating reduction of nitrates to volatile products, like nitric oxide (NO), nitrous oxide (N_2O) and in the end molecular nitrogen (N_2). The denitrification was described for a large series of bacteria (Spearl and Hoare, 1971; Pichinoty, 1973; Pichinoty et al., 1977; Zuhft and Cardenas,1979). The nitrates play the role of electron acceptors, reducing in successive stages, according to the scheme below:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

2. MATERIAL AND METHODS

The collected water samples. Unpurified water samples have been collected (from influent) from basin no. 1 and those of purified water (from effluent) from storage basin 2.

Media used. *Media 1* (nitrate-agar media) had the following composition (g/l): K_2PO_4 = 0.8; KH_2PO_4 = 0.4; NH_4NO_3 = 0.5; $MgSO_4$ = 0.1; $FeCl_3$ = 0.01; KNO_3 = 5.0; peptone = 1.0; glucose = 1.0; sodium acetate = 1.0; agar = = 18.0; water to 1.0 l; pH = 7.2 – 7.4; sterilisation 20 min at 121°C.

REV. ROUM. BIOL. – BIOL. ANIM., TOME 44, N° 2, P. 197–202, BUCAREST, 1999

Media 2 had the following composition (g/l): KH_2PO_4 = 0.5: NH_4NO_3 = 0.5; $MgSO_4$ = 0.1; $FeCl_3$ = 0.01: CuSO4 = =0.....0001; the tested nutrient (for instance acetate) = 5.0: water to 1 l; pH = 77.2; sterilisation 20 min. at 121°C.

Analytical methods. The test with the help of Durham tubes makes evident if studied bacteria use certain substrate (for instance acetate) in order to reduce to NO_3^-, CO_2 and N_2. It is used medium 2, in tubes (5–10 ml/tube). In these test tubes with medium, the tubes Durham are put inside down. They are inoculated for 72 h at the temperature of 28°C. If CO_2 and N_2 are accumulated, these getting to the surface of the medium in tubes, it means that the test is positive.

The molecular nitrogen test has been made with the help of a Packard gaschromatograph installation. In the atmosphere of the culture vessel, the air is replaced by CO_2, then it is incubated 72 h at 28°C. After incubation the gas in the atmosphere of the culture vessels is analysed at a gaschromatograph having a thermoconductibility detector. The presence of the molecular nitrogen shows that the cultivated bacteria realize a complete denitrification.

The analytical test with α-naphthylamine. This test is realized with the following reagent: reagent A (contains 0.5 α-naphthylamine dissolved in 100 ml 30% acetic acid); reagent B contains 0.8 g sulphanilic acid dissolved in 100 ml 30% acetic acid) and reagent C, powder of zinc. The application of the test was made in the following way. A test tube with 5 ml medium has been used in which the studied bacterium has been inoculated and thermostated for 3 days at 28 °C. After inoculation 0.5 ml reagent A and 0.5 ml reagent B have been added. If the medium turns into red, it means that it contains nitrite coming from nitrate reduction, and if it does not, then powder of Zn is added. If the medium in cultivated test tube with the studied bacterium turns into red in the presence of Zn. it means that the test tube contains nitrate and in this case the bacterium does not realize the denitrification. If the test tube does not turn into red, even after Zn addition, then nitrates have been completely removed, being reduced at molecular nitrogen, and as a result, the studied bacterium is able to realize a complete denitrification.

3. RESULTS AND DISCUSSIONS

The results obtained by experiments and microbiological analyses are presented in Tables 1, 2 and 3. In Table 1 are shown the results of the microbiological analyses regarding the presence (density) of the main physiological types of bacteria in the wastewaters at the Bucharest-Glina purification plant, both at the beginning of the purification period (in influent) and at the end of the purification period (in effluent). According to the results presented in Table 1, the total heterotrophic bacteria are found in influent at the densities of 10 000 000 bacteria / ml. After the purification period, their number decreases at

50 000 cells /ml in effluent, by floculation, sedimentation or even lysis of the bacteria involved in purification. The denitrifying heterotroph bacteria show cellular densities of 200 000 cells/ml (in influent) and 8 000 cells/ml in effluent. These data show that in both types of water, the density of the denitrifying bacteria is about 100 times more reduced than the density of the total heterotroph bacteria density. The nitrifying bacteria (ammonium-oxidative and nitrite-oxidative bacteria) show their reduced and stable cellular densities (2 000 respectively 500 cells/ml). The denitrifying autotroph bacteria of *Thiobacillus denitrificans* type present very low density values, under 100 cells/ml.

Table 1

Microbiological analysis of the domestic wastewater samples at Bucharest-Glina purification plant

Physiological bacterial group	Influent (bacteria/ml)	Effluent (bacteria/ml)
Total heterotroph bacteria	10 000 000	50 000
Denitrifying heterotroph bacteria	200 000	8 000
Ammonium-oxidative autotroph bacteria	2 000	500
Nitrite-oxidative autotroph bacteria	2 000	500
Sulph-oxidative autotroph bacteria (*Thiobacillus denitrificans*)	Under 100	Under 100

Table 2

The bacterial genera identified in the domestic wastewater at Bucharest-Glina purification plant

No.	Influent (bacterial genera)*	Effluent (bacterial genera)*
1	*Pseudomonas*	*Pseudomonas*
2	*Micrococcus*	*Micrococcus*
3	*Paracoccus*	*Paracoccus*
4	*Flavobacterium*	*Flavobacterium*
5	*Alcaligenes*	*Alcaligenes*
6	*Bacillus*	*Bacillus*
7	*Proteus*	*Nitrosomonas*
8	*Klebsiella*	*Nitrobacter*
9	*Enterobacter*	
10	*Citrobacter*	
11	*Arthrobacter*	
12	*Mycobacterium*	
13	*Nitrosomonas*	
14	*Nitrobacter*	

*Only the bacterial genera with densities higher than 100 cells/ml have been taken into consideration.

Bacterial genera identified in these water types are written in Table 2. The data in this table show that in the influent of the Bucharest-Glina purification plant, 14 different bacterial genera have been identified. In the effluent of the mentioned purification plant only 8 different bacterial genera have been identified, 6 genera contain polyvalent heterotroph bacteria and 2 genera contain monovalent denitrifying bacteria able to use a single metabolic mechanism in order to get the energy for growth and cellular multiplication. A program of "screening" has been used to select bacteria having high capacity of denitrification. These have been preserved under sterilized paraffin oil. The preserved bacteria are in Table 3.

Table 3

The bacteria with high capacity of denitrification, isolated from wastewater of the Bucharest-Glina purification plant, tested and preserved in the laboratory

No.	Bacterial strain	Isolation source	Repetition
1	*Pseudomonas spT_1*	Polluting soil	3
2	*Pseudomonas spT_2*	Polluting soil	3
3	*Pseudomonas spT_3*	Polluting soil	3
4	*Pseudomonas spT_5*	Polluting soil	3
5	*Pseudomonas spDC_1*	Domestic water	3
6	*Pseudomonas spDC_2*	Domestic water	3
7	*Micrococcus spT_1*	Polluting soil	3
8	*Micrococcus spT_2*	Polluting soil	3
9	*Micrococcus spT_4*	Polluting soil	3
10	*Micrococcus spT_5*	Polluting soil	3
11	*Micrococcus spDC_1*	Domestic water	3
12	*Micrococcus spDC_2*	Domestic water	3
13	*Micrococcus spM_1*	Activated sludge	3
14	*Micrococcus spG*	Domestic water	3
15	*Alcaligenes spDC1*	Domestic water	3
16	*Bacillus spDC_1*	Domestic water	3
17	*Flavobacterium spAB_1*	Domestic water	3
18	*Unidentified Gram-negative bacteria*	Domestic water	3

The results presented in Table 3 prove that 18 bacterial strains having an increased capacity of denitrification have been isolated, tested and preserved. Comparing the results in Table 2 with the ones in Table 3 it has been noticed that bacterial strains from genera with bacteria able to realize a complete denitrification, getting to carbon dioxide and molecular nitrogen, have been selected and preserved. The most selected strains belong to *Pseudomonas* and *Micrococcus* genera. There is a bacterial strain from *Flavobacterium, Alcaligenes, Bacillus* genera and an unidentified Gram-negative bacterial strain. In some older papers (Allen and van Niel,1952) it is shown the role of some denitrifying bacteria

such as those presented in this paper (*Pseudomonas, Micrococcus*). Pichinoty (1973) and also Pichinoty et al. (1978) show the role of bacteria in the reduction of nitrates, using mainly *Bacillus licheniformis*. The capacity of some bacteria in *Pseudomonas* genera to realize complete denitrification was also proved by Pichinoty et al. (1978). In the end, it can be said that in the waters used by us, there have not been identified strains of *Hyphomicrobium,* very often used in denitrification by some authors (Spearl and Hoare, 1971).

4. CONCLUSIONS

1. The total heterotrophic bacterial density level was about 10 000 000 cells/ml in influent and 500 000 cells/ml in effluent in domestic waste water at Bucharest-Glina purification plant.

2. The denitrifying heterotrophic bacteria had their density level about 100 times lower than the total heterotrophic bacteria in the influent (200 000) and also in the effluent (8 000).

3. Nitrifying bacteria (nitrate-bacteria and nitrite-bacteria) had their density level low and constant (about 2 000 cells/ml).

4. The denitrifying autotrophic bacteria like *Thiobacillus denitrificans* have sporadically been found and only at low densities (under 100 cells/ml).

5. Eighteen bacteria strains with high denitrification capacity have been isolated, selected and preserved in the study collection of the Microbiologic Center in the Institute of Biology of the Romanian Academy.

6. In the effluent of the Bucharest-Glina purification plant, the denitrifying heterotrophic bacteria like *Pseudomonas, Flavobacterium, Paracoccus* prevail and some others like: *Klebsiela, Enterobacter, Citrobacter, Proteus* are missing.

Acknowledgements. We thank ECOIND (ICPEAR) Institute, Bucharest, for helping us to collect the wastewater samples from the Bucharest-Glina purification plant.

REFERENCES

1. Alleman J.E.. 1984, *Elevated nitrite occurrence in biological waste water treatment systems,* War. Sci.Technol., **17**, 409–419.
2. Allen M.D. and van Niel C.B.. 1952, *Experiments on bacterial denitrification,* J. Bacteriol.. **64**, 397–412.

3. Amar D., Partos, J., Granjet, C., Faup, G. M., Audic, J.M.,1986, *The use of an up-flow fixed bed reactor for treatment of a primary settled domestic sewage,* Wat. Res., **20** (1), 9–14.
4. Pichinoty F., 1973, *La réduction bactérienne des composés oxigénés minéraux de l'azote,* Bul.Inst. Pasteur, Paris, **71**, 317–395.
5. Pichinoty F., Mandel M.,Greenway B. et Garcia J. L., 1977, *Étude de 14 bactéries dénitrificantes appartenant au groupe* Pseudomonas stutzeri *isolées du sol par culture d'enrchissement en présence d'oxyde nitreux,* Ann. Microbiol.(Inst.Pasteur), **128 A**, 75–87.
6. Pichinoty F., Garcia J.L., Job C. and Durand M.,1978, *La dénitrification chez* Bacillus licheniformis,Canad. J. Microbiol., **24**, 45–49.
7. Shanon J.R.M., and Unteritan R., 1993, *Evaluating bioremediation. Distinguishing facts from fiction,* Ann. Rev. Microbiol., **47**, 715–738.
8. Spearl G.T. and Hoare D.S., 1971, *Denitrification with methanol: a selective enrichment for* Hyphomicrobium species, J. Bacteriol., **10**(8), 733–736.
9. Zuhft W. G. and Cardenas J., 1979, *The inorganic biochemistry of nitrogen bioenergetic processes,* Naturwissenschaften, **667**, 81–88.

Received October 11, 1999.

Institute of Biology,
Spl. Independenţei 296,
PO-BOX 56–53, Bucharest, Romania

AMOEBIASIS CORRELATED WITH SOCIO-ECONOMIC FACTORS IN TWO AREAS OF YEMEN

AL SAKKAF GALAL*, IRINA TEODORESCU**

The investigation made between November 1998–August 1999, in urban and rural area from the Republic of Yemen, among a group of 1,204 patients, of different ages, professions, education level, family size, family income, source of drinking water, and of both sexes. The obtained data indicate that amoebiasis prevalence rates are inversely proportional to education level, family income (social level), and directly proportional to family size. In relation with age group, prevalence rate of amoebiasis is low among the infants 0–1 year, but increases among the children, 1–4 and 5–14 years old respectively, and then decreases at the age of 45 years. No significant differences in relation with sex.

1. INTRODUCTION

Amoebiasis is an endemic disease with etiologic agent *Entamoeba histolytica*, transmitted from man to man, through faecal-oral contamination, infested food handlers, homosexual men, etc. The prevalence of amoebiasis is possibly correlated to the low socio-economic conditions, below standard residences, poor hygienic and different climatic factors. The infestation exists nearly in most countries of the world, although usually it is considered as a tropical disease and no doubt the frequency is higher in countries with warm and humid climates, but it has also been observed in nearly every altitude.

The knowledge of amoebic infestation caused by the *Entamoeba histolytica* in Yemen is scanty particularly in the Southern and Eastern Governorates. The need to evaluate the risk of the disease and the most important socio-economic factors determinant to its prevalence, where this study is done including a large number of patients in comparison with other studies carried on years ago (1,2).

2. MATERIAL AND METHODS

Study period: November 1998-August 1999.

Study area: Aden with ≈ 441.900 people ("urban area"), is the commercial and economic capital of the Republic of Yemen. Most of the citizens work as employees in trade, fishing, and other manual professions. **Lahj** is a "rural area", placed at 30 km away from Aden, where the majority of the people work in agriculture.

Stool examinations, for patients referred from different policlinics were made at Al. Gamhoria Teaching hospital laboratory and at the Bin-Khaldon

hospital and the health centres near Al-Hotta (Lahj centre) and those from neighbouring villages.

A group of 1,204 patients (602 in Aden and 602 in Lahj), of different age groups were selected that complained with abdominal pain, with or without diarrhoea.

The parameters investigated were age groups, sex, occupations (nature of work experienced by the father or the person under investigation), businessmen (includes all types of commercial work), employees (includes all workers in different fields managed by the government), farmers, house-wives and other manual professions, fishing etc., **education level** (referring to the level of study, and for small age groups, the father's educational level), **family size**, **family income**, **source of drinking water:** tap water, (chlorinated sometimes, reaching to the consumers through common installation), unprotected wells, common big tanks (carried either by donkeys, camels or vehicles, then given to the consumers in their own containers), or streams (running water).

Methods: direct coproparasitologic methods (with physiological saline, and with Lugol's iodine), culture method Löffler medium (Simic method), cultivation using medium with spleen, Staining methods (with methyl blue, with Quensel-Svenson stain, Turdyev's method for amoebas differential diagnosis), the Questionnaire method (information obtained by the interview).

3. RESULTS AND DISCUSSION

Trophozoites and cysts characteristics observed: size, shape, pseudopodia, cytoplasm, granular aspect of endoplasm, vacuoles, nucleus, karyosome, chromatin granules arranged in the inner surface of the nuclear membrane, number of nuclei, chromatin bodies or erythrocytes in the cytoplasm, trophozoit motility.

Prevalence rate of amoebiasis in relation with sex (Table 1) showed males are more infested than females, in both areas, but the differences are not significant (15.9 %: 13.9%).

Prevalence rate of amoebiasis in relation with age group (Table 2). In Aden area, the prevalence rate increased with age, until the age of 5–14 years (up to 30 %), and then decreased towards the age of 45 years (under 5 %); small infants were also less infested (about 5 %). In Lahj area, the prevalence rate is lower in the infants 0–1 year (3.2 %), but increased among the children of 1–4 and 5–14 years old respectively. In both areas a greatest prevalence level is among children within 5–15 years old (28.5% and 31%).

Prevalence rate of amoebiasis in relation with education level (Table 3). In the two areas (Aden and Lahj) high prevalence rates among illiterates (over 47 %).

low prevalence rates among persons with university level (under 3 %) and with secondary school (under 10 %).

Table 1

Distribution of amoebic infestation by patient's sex

PARASITISM	MALE		FEMALE	
	No.	%	No.	%
URBAN AREA				
With Parasite	92	30.57	78	25.91
No Parasite	209	69.43	223	74.09
Total	301	100.0	301	100.0
RURAL AREA				
With Parasite	99	32.90	89	29.56
No Parasite	202	67.10	212	70.44
Total	301	100.0	301	100.0
BOTH AREAS				
With Parasite	191	31.72	167	27.74
No Parasite	411	68.28	435	72.26
Total	602	100.0	602	100.0

Table 2

Distribution of amoebiasis infestation by patient's age groups

AGE GROUP	ADEN infested	ADEN %	LAHJ infested	LAHJ %	BOTH AREAS infested	BOTH AREAS %
0 -1	9	5.3	6	3.2	15	4.2
> 1 - 4	49	28.8	53	28.2	102	28.5
5 - 14	51	30.0	60	31.9	111	31.0
15 - 24	25	14.7	28	14.9	53	14.8
25 - 34	18	10.6	23	12.0	41	11.5
35 - 44	10	5.9	12	6.4	22	6.0
≥ 45	8	4.7	6	3.2	14	4.0
	Total 170	Mean value 28.2	Total 188	Mean value 31.2	Total 358	Mean value 29.7

Prevalence rate of amoebiasis in relation with family size (Table 4). In Aden and Lahj areas, patients coming from large family members showed higher prevalence rates (over 53 %) and patients coming from a few family members showed lower prevalence rates (over 46 %); however, the differences are of little significance.

Prevalence rate of amoebiasis in relation with occupation (Table 5). In Aden area, the percentage of infested persons working in the business field was

low (about 14 %), comparative with the employees in different fields, managed by state (which was about three times greater). In Lahj area, also a higher percentage of infested persons was among employees (42 %), farmers (over 26 %) and house-wives (over 16 %). In both areas, a high level of infestation was among the same category (77 %), and a low infestation level among persons working in trade and commerce (14 %).

Table 3

Correlation of amoebic infestation with patient's education level

EDUCATION	URBAN AREA		RURAL AREA		BOTH AREAS	
	No.	%	No.	%	No.	%
Literates	43	25.3	38	20.2	81	21.0
Primary school	32	18.8	32	17.0	64	17.9
Secondary school	15	8.8	19	10.1	34	9.5
University	7	4.1	3	1.6	10	2.8
Illiterates	73	42.9	96	51.0	169	47.2
Total	170		188		358	

Table 4

Correlation of amoebic infestation with patient's family size

FAMILY SIZE	URBAN AREA		RURAL AREA		BOTH AREAS	
	No.	%	No.	%	No.	%
1 - 3 members	79	46.5	87	46.3	166	46.4
4- < 13 members	91	53.5	101	53.7	192	53.6
Total	170		188		358	

Table 5

Correlation of amoebic infestation with patient's occupation

OCCUPATION	URBAN AREA		RURAL AREA		BOTH AREAS	
	No.	%	No.	%	No.	%
Business men	24	14.1	26	13.8	50	14.0
Employees	75	44.1	79	42.0	154	43.0
Farmers	13	7.6	50	26.6	63	17.6
House-wives	28	16.5	31	16.5	59	16.5
Others	30	17.6	2	1.1	32	8.9
Total	170		188		358	

Prevalence rate of amoebiasis in relation with family income (social level) (Table 6). High prevalence of amoebiasis registered among persons with

low and intermediate monthly incomes (over 79 % in urban area, and about 90 % in rural area). Low prevalence rates among families having higher monthly income (over 20 % in Aden and over 10 % in Lahj).

Table 6

Correlation of amoebic infestation with patient's social level (family income)

FAMILY INCOMES IN Y.R/ MONTH	URBAN AREA		RURAL AREA		BOTH AREAS	
	No.	%	No.	%	No.	%
High > 20000	35	20.6	20	10.6	55	15.4
Intermediate	65	38.2	53	28.2	118	33.0
Low < 20000	70	41.2	115	61.2	185	51.6
Total	170		188		358	

Table 7

Correlation of amoebic infestation with quality of drinking water

SOURCE OF DRINKING WATER	URBAN AREA		RURAL AREA		BOTH AREAS	
	No.	%	No.	%	No.	%
Tap water	78	45.9	11	5.9	89	24.8
Wells	–	–	122	64.9	122	34.1
Common tanks	92	54:1	10	5.3	102	28.5
Streams	–	–	45	23.9	45	12.6
Total	170		188		358	

Prevalence rate of amoebiasis correlated with the quality and source of drinking water (Table 7). In Aden area, a high prevalence rate of amoebiasis among persons using the only two water sources for drinking and washing purposes (common tanks and tap water). The high percentage was among those using common tanks, where contamination may take place during transportation and distribution to consumers. In Lahj area, a high prevalence rate of amoebiasis among persons using unprotected sources to obtain water, especially wells.

4. CONCLUSIONS

- No significant differences of amoebiasis prevalence with patient's sex;
- Prevalence increases with age until 15 years old, then decreases towards the 44 years old persons;

- Prevalence rates are inversely proportional with education level;
- The number of infested persons living in crowded families is higher, than the number of infested persons living in less crowded families;
- Low prevalence rate among persons working in the business field and housewives, but high prevalence among employees and farmers;
- The great number of infested persons, come from families with low monthly income;
- High prevalence of amoebiasis registered among persons using unprotected sources of water for drinking and washing purposes.

REFERENCES

1. Gamal Z., *Prevalence of intestinal infestation among children in Yemen,* A thesis for PhD., 1996.
2. Kopecky K., Giboda M., Aldova E., Dobahiss and Radkovsky J., *Pilot studies on the occurrence of some infectious diseases in two different areas in South Yemen (Aden), Part I, Parasitology,* Journal of Hygiene, Epidemiology, Microbiology & Immunology, vol **36**, nr. *3*, 253–261, (1992).

Received September 20, 1999.

*Faculty of Medicine, Aden University, Yemen
** Faculty of Biology, Bucharest University, Romania

THE DETERMINATION OF IRRITATION POTENTIAL OF BIOPOLYMER OF *XANTHAN* TYPE ON RABBITS

MARIOARA FINTA-ISTRATE*, ANCA VOICU**, I. LAZĂR**

The biopolymer of *xanthan* type has several biotechnological applications in industries such as: petroleum, chemistry, chemical-pharmaceutical, food, textile, cosmetics, agriculture and environment protection. This biopolymer is produced by bacteria of genus *Xanthomonas*.

For the first time in Romania, this type of biopolymer was studied and produced at the Institute of Biology of the Romanian Academy. It was considered of interest to know more about this bacterial biopolymer from the toxicity and pathogenity point of view. Thus, investigations were done concerning the local tolerance test, using an acute experiment for establishing "the Primary Dermal Irritation Index" (PDII) and a chronic experiment for the assessment of the superficial cutaneous aggressive action. The experiments were carried out on rabbits.

It was found that the PDII value is 0.06, which means a local cutaneous tolerance in normal value limits, and it is in accordance to the lack of superficial cutaneous aggressive action. The lack of pathological modifications proves that the tested *xanthan* biopolymer has no toxic action.

1. INTRODUCTION

There are many biotechnological applications of polymers of biosynthesized exopolysaccharides type such as: petroleum, chemistry, chemical-pharmaceutical, food, textile, cosmetics, agriculture and environment protection [13]. At international level, the dynamic extension of biotechnological applications based on the use of microorganisms imposes an evaluation of the potential effects on the health status of people who handle such microorganisms or their products [7]. The United States Environmental Protection Agency and Federal Register statutes regulating the release of microbiological agents mandate that the microorganisms used not to be pathogenic. However, because of the increased exposure potential and possibility for the use of identified or unknown opportunistic pathogenic agents, it is imperative to determine if a microorganism has the probability to infect workers or the general public [15,17]. Human may come into contact with these microorganisms or their products in the industrial setting during production or application. Because soil, water or air may contain high concentrations of the microbial product, the primary routes of exposure are through inhalation, ingestion, or skin penetration [5,10,16,18]. Therefore, several *in vivo* and *in vitro* systems have been used to determine if biotechnological agents and their products are potentially hazardous making tests such as: toxicological tests which address acute oral, dermal, pulmonary and subchronic toxicity/pathogenity , oncogenicity studies, primate pathogenity studies [1,6,14,17].

REV. ROUM. BIOL. – BIOL. ANIM., TOME 44, N° 2, P. 209–214, BUCAREST, 1999

At national level, a research team from the Microbiological Department of the Institute of Biology of the Romanian Academy (Bucharest) used the microbiological method studying the biopolymer of *xanthan* type in different biotechnological applications [12,22].

This paper is a part of a larger study concerning the pathogenic and toxic action of some bacterial cultures and bacterial products (biopolymers, biosurfactants) which are used in biotechnological processes.

The purpose of the experiment described in this paper is the assessment of the toxic action of the biopolymer of *xanthan* type, through the evaluation of irritation potential and/or of corrosive effects to rabbits. The local tolerance test, including an acute experiment (the determination of the Primary Index of Dermal Irritation - PDII) and a chronic experiment (the assessment of the superficial cutaneous aggression), intended the emphasizing of the alterations at the site where the inoculum was administered.

2. MATERIALS AND METHODS

The tested biopolymer. The exopolysaccharide of *xanthan* type is a bacterial product known with different commercial names, such as: *kelzan, ketrol, rhodigel. rhodopol, flocon xanflood,* etc. The microorganism which produces this type of biopolymer is *Xanthomonas campestris* (from cultures collection of the Microbiology Department of the Institute of Biology, Bucharest). Concerning the chemical composition, the bacterial product is composed [8,9,21] by monosaccharides and organic acids (glucose, mannose, glucuronic acid. pyruvate acetyl).

The polysaccharide used as test material being a solid material, it was moistened with physiological saline, according to the used protocols [2,20]: the quantity of applied solid material must be of maximum 0.5 cm^3.

Animals. 15 rabbits for the chronic experiment and 5 rabbits for the acute experiment were selected and acclimated. The animals (of both sexes: 10 female, 5 male), which belong to the „common rabbit" breed, were not used for any previous experimental procedures; females were nulliparous and nonpregnant [3].

18 to 24 hours prior to topical administration of the test material, the hair of each rabbit in the dorsal and lateral trunk areas was closely clipped with an electric clipper skin. In the first and the last day of the experiment, the body weights of the animals were determined and recorded.

Inoculation. A 0.5 g sample of the tested biological material represented by polysaccharide (solid test material) was applied beneath the gauze patch which was secured to the appropriate application site. The test article patch was then moistened with physiological saline by administering 0.5 ml to the outside surface of the

secured patch. The patches were covered with an occlusive dressing consisting of an impervious material for the duration of the exposure period. The occlusive dressing was secured with tape, especially at the anterior and posterior ends, to retard evaporation. As reference, a negative control patch with physiological saline and a positive control patch with the aqueous 1% w/v sodium lauryl sulfate solution were used. The negative and positive control patches were applied (0.5 ml) to the appropriate application site in the manner described for the tested material.

Table 1

Draize's grading system for evaluation of dermal reactions

Erythema and eschar formation		**Edema formation**	
Dermal reaction	**Score**	**Dermal reaction**	**Score**
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well defined erythema	2	Slight edema (edges of real well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond exposure)	4
Total possible erythema score	4	Total possible edema score	4

Table 2

Interpretation of results

Codes for other dermal effects		**PDII (Primary Dermal Irritation Index)**	
Code	**Dermal effect**	**PDII Value**	**Interpretation**
N	Necrosis	0.0	Nonirritant
E	Eschar	0.0 – 0.5	Negligible irritant
T	Thickening	0.5 – 2.0	Mild irritant
B	Blanching	2.0 – 5.0	Moderate irritant
F	Fissuring	5.0 – 8.0	Severe irritant
D	Desquamation	8	Maximum possible calculated PDII

After approximately 24 hours of exposure, the dressing and gauze patches were removed. Immediately after removal of patches each square application site was outlined with an indelible marker by dotting each of the four corners to delineate to

sites for subsequent identification. Residual test material was wiped (but not washed) with gauze sponges moistened with physiological saline without altering existing response or the integrity of the epidermis.

In the chronic experiment, concerning the assessment of the superficial cutaneous aggressive action, the administration of the biopolymer was carried out by multiple applications during 30 days. In the case of the 5 females of rabbits, daily applications were done in conditions similar to those of the acute experiment.

All the alterations of the skin were scored for dermal irritation according to the Draize scoring system (refer to *Table 1*) and the interpretation of results system [4] (refer to *Table 2*), at approximately 1, 24 and 48 hours after removal of wrapping (25, 48 and 72 hours after the initial administration).

3. RESULTS AND DISCUSSION

The reactivity of the animal model depends on its organic predisposition and on its physical and chemical structural relations. Taking into account the physical properties of exopolysaccharide and the substrate of cutaneous mechanism of absorption, it was found a concordance between the estimated and experimental results.

Thus, in concordance with the protocol, PDII is calculated by summing the mean erythema scores and the mean edema scores from only the 25–. 48– and 72–hours observations and for the test material site, the positive control site and the untreated site. In our experiment, after 48 hours the erythema scores were 0.2 for 3 rabbits and 0 for 7 rabbits. The edema scores were 0 for all the rabbits. The value of PDII was 0.06. Comparing the PDII value with the interpretation of results system (Draize) it was found that this value is included in the PDII interval 0.0–0.5, which means: negligible irritant. The erythema represented the single cutaneous pathological manifestation in this experiment, evaluated at 30% percent and having a very small intensity. The dermal aspect of reference areas (positive, negative) confirms the specificity of reaction. Taking into account that the cutaneous reactions are of allergic nature, it is important to consider thoroughly the allergenic mechanism which is related to them. Thus, some studies [18] classify the allergic reaction in a second category, after pathogenicity, of human exposure consequences to some biotechnological agents and/or their products.

In this experiment, the erythema was established at 2 females and 1 male. and consequently, the sex factor is considered to have no relevance. Also. it was confirmed the lack of toxicity of the bioplymer of *xanthan* type. The values of the body weights recorded on the first day of the experiment for the animal models were compared to those recorded on the last day (after 72 hours); the daily mean growth. resulted from this evaluation, was 50.51 g/rabbit/day. The vital functions happen in

normal value limits. During the chronic experiment, it was established that there are not significant cutaneous reactions, either local or general.

4. CONCLUSIONS

1. Observing the weight evolution of rabbits and the daily mean growth (50.51 g/rabbit/day), it was found that the assimilation processes were not affected.

2. In this experiment, the sex factor has no relevance.

3. The erythema was the only pathological manifestation of the cutaneous reaction in this experiment, observed at 30% of the animal models, but at a very weak intensity.

4. The Primary Dermal Irritation Index (PDII) was 0.06, which means a local cutaneous tolerance in normal value limits. The absence of the pathological changes when the biopolymer was maintained for a long time in contact with the skin certify the lack of toxicity for this bacterial product.

5. The tested material, represented by the biopolymer of *xanthan* type, produced by bacteria of species *Xanthomonas campestris,* has no toxic effects.

REFERENCES

1. Claxton L.D.. Houk V.S., Williams R. Kremer F.. *Effect of bioremediation on the mutagenicity of oil spilled*, Priced Wiliam Sound, Alaska. **23**: 643–650 (1991).
2. Derelanko J.M.. *Protocols*, Dabt Dept. of Toxicology Allied – Signal Inc., Engineered Materials Sector. P.O. Box 1139, Morristown. N.J. (1994).
3. Dima S., *Imbreed lines in the biomedical increase*. Biology of animal models and comparative oncology (in Romanian), 19 (1992).
4. Draize J.H., *Dermal Toxicity*, Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, The Association of Food and Drug Officials of the U.S.A., Austin, Texas, 46–48 (1959).
5. George S.E.. Kohan M.J., Taylor M.S.. Brooks H.G., Creason J.P., Claxton L.D., *Intestinal survival, competition and translocation of biotechnology agents on intranasal exposure of C3H/HeJ mice,* Environmental Toxicology and Chemistry, **13**: 1145-1152 (1994).
6. George S.E.. Kohan M.J., Whitehouse D.A., *Distribution, clearance and mortality of environmental pseudomonads in mice upon intranasal exposure*. Applied and Environmental Microbiology, **57**: 2420–2425 (1991).
7. Gofrey A.. Cambell M., Lam J.. Paranchych W.. Speert D.. Wood D.E *Current research priorities pertaining to health requirement for biotechnology products under Canadian Environmental Protection Act.*, Proceedings of the Seventh Annual Symposium on Environmental Release of Biotechnology Products: Risk Assessment Methods and Research Progress (eds LEVIN M.), U.S. Environmental Protection Agency/U.S. Department of Agriculture/Environment Canada University of Maryland Biotechnology Institute. College Park. M.D.. 211–227 (1996).
8. Jansson E.P.. Kenne L., Lindberg B.. *Structure of the extracellular polysaccaride from Xanthomonas campestris*. Carbohydrate Research. 275–282 (1975).
9. Jeanes A.. Rogovin P., Cadmus M.C., Silman R.W.. Knutson C.A., *Polysaccharide (xanthan) of pure culture. maintain and polysaccharides production. purification and analysis,* Agricultural Research Services U.S. Dept. of Agriculture. **ARS-nc-51**: 1–14 (1976).

10. Kawanishi C.Y., George S.E., Sherwood R.L., *Health effects assessment of pulmonary exposure to biotechnology agents,* Review of Progress in the Biotechnology - Microbial Pest Control Agent Risk Assessment Program, **EPA/600/p-029**: 214–215 (1990).
11. Kaiser A., Classen H.G., Eberspacher J., Lingens F., *Acute toxicity testing of some herbicides-alkaloids-, and antibiotics-metabolizing soil bacteria in the rat,* Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene, **B173**: 173–179 (1981).
12. Lazăr I., Grigoriu A., *A proceeding to produce the xanthan type of biopolymer, Certificate of innovator nr. 84 (1984).*
13. Paul F., Morin A., Monson P., *Microbial polysaccharides with actual potential industrial applications*, Biotechnology Advances, 4 (2): 245–259 (1986).
14. Sayler G.S., Sayre P., *Risk assessment for recombinant pseudomonads released into the environment for hazardous waste degradation,* Bioremediation: the Tokyo 1994 Workshop, Org. of Ec. Cooperation and Develop., Paris, 263–272 (1995).
15. Seidler R.J., Watrud L.S., George S.E., *Characterizing ecological effects,* Handbook of environmental risk assessment and management, Blackwell Science Ltd., USA, 121–122 (1998).
16. Sleider R.J., Wadrut L.S., George S.E., *Health effects,* Handbook of environmental risk assessment and management, Blackwell Science Ltd., USA, 123–125 (1998).
17. Sleider R.J., Wadrut L.S., George S.E., *Toxicological consideration, Tier testing approach for microbial and biochemical agents*, Assessing Risks to Ecosystem and Human Health from Genetically Modified Organisms, 131–134 (1998).
18. Sleider R.J., Wadrut L.S., George S.E., *Allergenicity,* Handbook of environmental risk assessment and management, Blackwell Science Ltd., USA, 126–128 (1998).
19. Simionovici M., Cârstea, Vlădescu C., *The absorption,* Pharmacological researches and drugs investigation (in Romanian), Medical Press, 288–294 (1983).
20. Simionovici M., Cârstea, Vlădescu C., *The local tolerance*, Pharmacological researches and drugs investigation (in Romanian), Medical Press, 436–439 (1983).
21. Taylor C.K., Nasr-El-Din A.H., *Xanthan biopolymers: a review of methods for the determination and for the measurement of acetate and pyruvate content*, Journal of Petroleum Science and Engineering, **9**: 273–279 (1993).
22. Voicu A., *Conservation in time of capacity of Xanthomonas strains to biosynthesize the biopolymer of xanthan type*, Industrial microbiology and biotechnology (in Romanian), Iaşi, 781–784 (1988).

Received November 1, 1999.

*Centre for Protection, Hygiene and Ergonomics in Professional Exposure S.A., Str. Pop de Băsești 59, Bucharest

**Institute of Biology of the Romanian Academy, Spl. Independenței, nr. 296, București, Romania

AVIS AUX COLLABORATEURS

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 plus récentes parutions dans la littérature.

Les auteurs sont priés de présenter leurs articles en double exemplaire imprimés, de préférence sur une imprimante laser et espacés à double interligne. Le contenu des articles sera introduit sur des disquettes dans un langage connu, préférablement Word 6.0. La composition et la mise en vedette seront faites selon l'usage de la revue: caractères de 11/13 points pour le texte, de 12/14 points pour le titre de l'article et de 9/11 pour les annexes (tableaux, bibliographie, explication des figures, notes, etc.) et le résumé en anglais de 10 lignes au maximum, qui sera placé au début de l'article. Il est obligatoire de spécifier sur les disquettes le nom des fichiers ainsi que le programme utilisé.

Le matériel graphique sera envoyé sur disquette, scanné, avec les mêmes spécifications. En l'absence d'un scanner, le matériel graphique sera exécuté en encre de Chine sur papier calque.

Les tableaux et les illustrations seront numérotés en chiffres arabes dans l'ordre de l'apparition. Les titres des revues seront abrégés conformément aux usages internationaux.

Les textes ne doivent pas dépasser 10 pages (y compris les tableaux, la bibliographie et l'éxplication des figures).

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