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CONTINENTS, OCEAN BASINS AND FRESHWATER ZOOGEOGRAPHY. II. SOUTH AMERICA AND AUSTRALIA/NEW GUINEA

PETRU M. BĂNĂRESCU

The tropical and cold-temperate South American faunas of primary freshwater animals and aquatic insects differ sharply, the former including many exclusive lineages shared with Africa, the latter lineages shared with Australia; there are however also lineages shared by both South American areas (most having Australian ties), while some taxa restricted to the temperate area have tropical South American affinities (especially among fishes). The differences between the temperate and tropical areas of Australia/New Guinea are less marked; many transantarctic lineages inhabit both halves of the Australian region. The primary freshwarter fauna of temperate Australia is more closely related to the tropical Australian/ New Guinea than to the South American one.

It has been demonstrated in a previous paper (6) that the distribution patterns of the lineages of primary freshwater animals and of aquatic insects in Africa contradicts the assertion of Croizat (11, 12) and of Parenti (33) according to which the ranges of lineages correspond to ocean basins rather than to continents/: the primary freshwater fauna of western Africa actually has much closer ties with that of eastern Africa than with that of tropical South America that lies on the opposite side of the Atlantic Ocean. Similarly, the primary freshwater fauna of eastern Africa is more closely related to the western African one than to that of southern Asia on the opposite site of the Indian Ocean. On the contrary, the east African fauna of peripheral freshwater animals has closer ties to the southern Asia, and the western African one to the South American one.

As in Africa, two main subdivisions can easily be distinguished within the freshwater fauna of South America: a tropical and a temperate/cold one. South America once had continental connections with Africa on the one hand, with Australia/New Guinea and New Zealand on the other hand; it is presently connected with North America through a narrow and geologically young landbridge, Central America.

Considering both recent geography and former continental connections, one would expect tropical South America to have close biogeographic ties with Africa and North America and temperature/cold South America with Australia/New Guinea.

Fig. 1. — Representation of the relationship of the Australian fauna of primary freshwater animals and of aquatic insects.

The primary freshwater fishes of tropical South America belong to six higher taxa; five of them are also present in Africa. Shared exclusively by both continents are Lepidosirenidae (one South American and one African genus) and Characoidae (thirteen South American families, a few of which also extend to Central and North America; the South Ame-

rican Characidae is the sister of the African Alestidae : [21]). Present in South America, Africa and southern Asia are Nandidae (two American genera) and the large suborder Siluroidei, with 12 families in tropical South America, four of which are closely related to African ones : 10), while Osteoglossidae is also present in tropical Australia/New Guinea, the South American *Arapaima* being the sister of the African *Heterotis* (32). Finally, the last tropical South/Central American lineage of primary freshwater fishes, the suborder Gymnotoidei is endemic ; it is presently considered the apomorphic sister of Siluroidei, (16) its affinities being hence African/south Asian, too.

It is worth mentioning that four of these six lineages are restricted in South America to the tropical area, Characiformes and Siluroidei alone containing a quite small number of species in the south.

A single genus of tropical South American primary freshwater fishes has Australian ties : *Osteoglossum*, whose closest related genus, *Scleropages*, ranges in tropical Australia/New Guinea and in South-eastern Asia (32).

Few genera of primary freshwater higher crustaceans inhabit tropical South America : *Hyalella* (amphipod), apparently without close relatives, *Nannobathaynella* (Bathynellidae) shared with Africa (42) and about five genera of Parabathynellidae, at least four of which belong to a lineage also in Africa and Madagascar (41).

Three families of large primary freshwater mussels occur in tropical South America, two of which are richly represented also in the temperate zone : restricted to the tropics is Muelleriidae, shared only with southern India (23) ; this widely disjunct range clearly demonstrates the former presence also in Africa.

The main lineage of primary freshwater prosobranchiate gastropods in the area, Pilidae is shared with Africa and tropical Asia ; the tropical South American genera of other lineages seem to have on the contrary North American ties : Pleuroceridae *(Doryssa* and *Lithasia :* 31), Lithoglyphinae *(Pomatolithus :* 14), the mexithaumine and littoridinine lineages of hydrobioids (44) while the only native genus of Pomatiopsidae, *Aquidauania* is systematically isolated, being considered the sister of a group of six genera distributed in South Africa, Australia, south and east Asia (13). Of the two local genera of pulmonate snails, *Drepanotrema* is the closest to an African and *Gundlachia* either to an African or to a North American genus (24).

Exclusive of tropical South America and of Africa is also a family of primary freshwater sponges, Potamolepidae (7), and a subfamily of oligochaetes (8). Richly represented in tropical South America is also the primary freshwater family Diaptomidae of calanoid copepods ; this comprises ten endemic South American genera, nine in the tropical, one in the southern cold area (15). These genera may be closer either to African or to North American ones ; the family is practically absent from Australia.

Nine genera of arctoperlarian Plecoptera inhabit tropical South America (43) ; all belong to the subfamily Acronerinae of Perlidae, that is mainly North American and is absent from Africa (43, 47). African ties also have the only lineage of blepharoceride midges from tropical South American Paltostomatini (one genus is shared with Africa, two are exclusively tropical South American, one Central American, the last one extends from subtropical to temperate South America : (48) and a single genus of caddis flies : *Leptonema* with numerous species.

Most other tropical South American genera of caddisfilies belong to widely ranging genera and subfamilies. Several have North American ties : numerous Hydroptilidae, (28), the two genera of Xiphocentronidae (39) ; however, *Etochorema*, confined to tropical South America and Middle America, belongs to a family (Hydrobiosidae) centering in Australia/New Guinea and southern South America (40).

Most lineages of primary freshwater animals confined to southern South America, have notogeic, above all Australian ties : the fish family Percichthyidae (two South American genera, eight species : the others in the south of Australia : 2), both families of primary freshwater decapod crustaceans (Parastacidae with two South American genera, each related to a south Australian one (37) and Aeglidae, endemic to temperate-subtropical South America, with fossil record also from New Zealand : 5), the family Stygocarididae of syncarid crustaceans (three genera in southern South America, the others in New Zealand and southern Australia, three related families in south-eastern Australia/Tasmania) : 26), the *Chilibathynella*-group of genera within the syncarid family Parabathynellidae (two genera in cold South America, shared with either Australia or New Zealand, another in tropical South America and one in southern Australia and New Zealand ; 41), the genus *Glacidorbis* of hydrobioid prosobranchiate snails (shared with Tasmania, south-eastern and south-western Australia : 30), the family Boeckellidae of calanoid Copepoda (shared mainly with southern Australia and New Zealand, isolated also in north-eastern Asia : 15), the entire suborder Antarctoperlaria of stoneflies (one family endemic to temperate South America, the three others shared with southern Australia/Tasmania and New Zealand, one of them extending also to subtropical South America : 47) and the family Notonemouridae (distributed in all southern continental areas, the genera from southern South America being more closely related to those from southern Australia and New Zealand than to those from southern Africa and Madagascar : 25), three circum-Antarctic families of caddisflies, shared with south Australia/Tasmania and with New Zealand (Rhynchopsychidae, Helicophidae), one only with Australia/Tasmania (Tasimiidae) (5), and the genus *Edwardsina* of net-winged or blephariceride midges (1, 48).

Most of these lineages are confined in South America to the southern, temperate or cold areas, the strong family Gripopterygidae alone extending to the subtropical, but not to the equatorial part of the continent (47), while Parastacidae and Aeglidae extend to the southern part of the Rio Paraná basin and to the coastal rivers of Rio Grande do Sul (37 ; 5).

Two large lineages of caddis flies shared with the Australian region and with New Zealand have a more complex distribution pattern : the family Hydrobiosidae (40) and the subfamily Triplectidinae (28). Both range throughout whole South America (the former even in the south of North America, too) as well as in tropical Australia/New Guinea, in South and East Asia : they are however more diversified in southern than in tropical South America. Another prevailing „Gondwanian" family, Ecnomidae, has a single Neotropical genus, *Austrotinodes*, that comprises a group

of species from Chile to Paraguay and another in Mexico-central America (18).

Endemic to cold-temperate South America are however also lineages having not trans-Antarctic, but tropical South American ties. These are numerous especially among primary freshwater fishes : the family Diplomystidae (two genera, five species : 3), which is considered the most plesiomorphic of the pan-tropical, prevailingly tropical order Siluriformes, the monospecific subfamily Nematogenyinae, the genera *Hatcheria* and *Bullockia* (Trichnycteridae, Siluriformes), the genus *Gymnocharacinus* (assigned to a distinct tribe : 21) and a monophyletic group of three species of Cheirodon (Characidae), besides some eight species of *Trichomycterus* (4, 21); this makes a total amount of about 20 species (12 being members of endemic supraspecific lineages), as against only eight species of the trans-Antarctic Percichthyidae. Tropical South American ties also have *Tumeodiaptomus* from a lake in Chile, far beyond the main range of Diaptomidae in the continent (15) and rather many canddisflies of the families Glossosomatidae (three endemic genera in a prevalently tropical-American subfamily : 17), Hydroptilidae (the endemic *Celaenotrichia* and a few species in other genera : 19 ; 28) and Polycentropodidae.

A special category is represented by the bipolar taxa, distribuited in the northern continents and in southern South America, or also the Andes : five or six endemic Chilean-Argentinean (or also Andean) genera of Limnephilidae (caddisflies) and seven species of the fairy shrimp genus *Branchinecta* (27) the others are Holarctic). These evidently dispersed along the Andes, e.g. through tropical South America. A bipolar range also has the caddis fly family Sericostomatidae ; with seven endemic genera in Chile-Argentina (19 ; two of the nine listed genera have been removed into another family) ; it is however not known if these are closer to the Holarctic or to the Australian genera ; in the latter case they must be ascribed to the trans-Antarctic lineages.

A few lineages are endemic to South America, being about equally represented in its two subdivisions. The principal ones are the two speciose groups of freshwater mussels : the family Mycetopodidae and the subfamily Hyriinae ; the former is the sister of the African Mutelidae the second the sister of two subfamilies from Australia and New Zealand ; one would expect Mycetopodidae to be restricted to the tropical areas, Hyriinae to the temperate and cold one ; actually, both have practically the same distribution, from the extreme north of the continent (Mycetopodidae even from Middle America) to Patagonia ; only on the Pacific slope do Hyriinae extend further south, in Chile (34). Another lineage endemic to the continent and present in both parts is the caddisfly family Anomalopsychidae (20) ; being a member of Sericostomatoidea, it probably has circum—Antarctic ties.

There is no sharp limit between the tropical and the temperate/cold subdivisions ; the aquatic fauna of the Rio Paraná basin is mainly tropical, but in its southern area, as well as in the coastal rivers of Uruguay and Rio Grande do Sul are also present some members of the temperate/cold lineages.

The tropical and southern faunas of primary freshwater animals and of aquatic insecta in South America are however more different than the east African and west African ones. It is not possible to quantify the differences between both and between the former and the African or the latter and the Australian fauna. Considering only the number of shared species and lineages, the fish fauna of southern South America seems to be more similar to the tropical South American than to the Australian one ; the freshwater invertebrate fauna is on the contrary more similar to the Australian one.

The tropical South American fauna of peripheral freshwater fishes, crustaceans and molluscs is a derivative of the tropical marine fauna of the Atlantic. Numerous species and genera within prevailingly tropical marine families are fully adapted to fresh water but none of them is known to have closer ties to the western African members of the same families. Close affinities with the western African inland water fauna have on the contrary many prawns (species of *Atya*, *Palaemon*, *Macrobrachium*, of Euryrhynchinae and Alphaeidae), snails (especially Neritidae) and mussels (shared genera restricted to the Atlantic slope of tropical America and Africa are *Congeria*, *Egeria Iphigenia*). It is worth mentioning however that the inland water fauna of peripheral animals in Africa, even in western Africa, is dominated by derivatives of the Indo-West Pacific, not of the tropical Atlantic marine fauna.

Most peripheral freshwater fishes from southern South America belong to trans-Antarctic families, also present in Australia and absent from the tropics : Geotriidae, Mordaciidae, Galaxiidae and Aplochitonidae ; tropical marine and South American ties have only the few inland water Atherinidae from Chile and southern Argentina. There are practically no southern peripheral invertebrates.

Australia and New Guinea have been connected until quite recent geological time and built a single biogeographic realm. This lies in the vicinity of south-eastern Asia but since the breakup of Pangaea, it has never had continental connections with Asia ; during Mezosoic times it was connected with the other southern or Gondwanian continents ; the last connection has been with South America, through the intermediary of Antarctida. It lies closer to New Zealand, but the direct continental connection ceased earlier. The aquatic fauna of Australia/New Guinea also shares a few lineages with Africa and Madagascar.

The most significant lineages of primary freshwater animals are shared with South America and are concentrated in the south of Australia. These are : Percichthyidae (fishes ; only the south of Australia and of South America), Parastacidae (crayfishes ; more diversified in the south of Australia, also in southern New Guinea : 37 ; 38), Hyriidae (mussels : shared with New Zealand and South America ; throughout the Australian region, including northern New Guinea : 29, 45), Glacidorbis (hydrobioid snails ; only in the southern areas of Australia and of South America ; 30) ; the *Chilibathynella*-lineage of Parabathynellidae (syncarid crustaceans ; south-eastern Australia, Chile and New Zealand : 41) ; the order Anaspidacea, again of syncarid crustaceans (same general range, three families being endemic Australian, the fourth one shared with temperate South America and New Zealand : 26) ; four families of stone flies (three in the order Antarctoperlaria, Notonemouridae in *Arctoperlaria* : 47) and four of caddis flies (Tasimiidae shared only with temperate South America, Kokiriidae, Philorheithridae and Helicophidae with temperate South

America and New Zealand as well : two other families are shared only with New Zealand and three endemic to south-eastern Australia, all having circum-Antarctic ties : 5), the genus *Edwardsina* of blephariccrid midges (exclusively in south-eastern Australia and south-western South America : 48) and Boeckellidae (calanoid Copepoda : 15). Most of these lineages are restricted in the Australian region to the southern half of the mainland and to Tasmania, Hyriidae and Parastacidae alone also ranging in the tropics (including New Guinea). To a similar category belong two important taxa of caddisflies (Hydrobiosidae and Triplectidinae : 40, 28), which are however also present in south and east Asia (and in Middle America and the south of the U.S.A.)

Brundin (9) and other authors have pointed out that the Australian members of various sub-Antarctic lineages are more closely related to the South American than to the New Zealand ones. This is also the case with several of the taxa mentioned above : Tasimiidae ranges only in Australia and South America ; three Australian genera of Parastacidae have their sisters in South America, a single one in New Zealand (37) ; four Australian genera of Hydrobiosidae belong to sub lineages also in South America and no sublineage is exclusively in Australia and New Zealand (40). In other cases, however, the Australian members are more closely related to New Zealand ones : for ex. in Hyriidae (two subfamilies in both areas, another in South America : 34) ; two families of caddis flies are exclusively Australia and New Zealand ; the stoneflies of the subfamily Stenoperlinae from Australia and New Zealand are congeneric, the South American ones belong to another genus : 47) etc. Shared exclusively by the Australian region (inclusively southern New Guinea) and New Zealand is the leech family Richardsonianidae, the species from south-eastern Australia being congeneric with those from New Zealand and more distantly related to those from other Australian districts (35 ; 36).

The aquatic fauna of Australia has trans-oceanic relations also with the African and Madagascan ones : the only Australian genus of Pomatiopsidae (snails), *Coxiella* (confined to the south and the west) is the sister of the south African *Tomichia* (13) ; the south-east Australian *Astacopsis* (Parastacidae) is the sister of the Madagascan *Astacoides*) (37). Trans-oceanic ties with south Africa and New Zealand have two lineages of peracarid crustaceans : the subfamily Ceininae (amphipods : the Australian species are closer to the African than to the New Zealand ones : 22) and the suborder Phreatoicidea (isopods ; two south-western Australian genera are closely related to the only south African genus, while several south-eastern Australian ones are close to New Zealand genera ; phreatoicids are also present in northern Australia : Dr. Knott, in litt.)

Few primary freshwater animal lineages are peculiar to tropical Australia : only three fish families (the endemic monospecific Ceratodidae with no relatives in the recent fauna ; Osteoglossidae with two species of *Scleropages* in north-eastern Australia/southern New Guinea, the third one in south-eastern Asia and the next relative in tropical South America (32) ; the speciose Melanotaeniidae — including Pseudomugilidae — with no freshwater relatives in south Asia or elsewhere). Two families of primary freshwater prosobranchiates are present, both shared with south Asia and Africa : Bithynidae (the only Australian genus *Gabbia*, ranging throughout the eastern half of the continent and in south New Guinea may be closest to an African one) and Viviparidae (two endemic genera in eastern, tropical to temperate Australia and the Afro-Asian *Bellamya* in New Guinea (5)). The fish fauna comprises numerous genera of marine origin, but fully adapted to freshwater ; most have no close ties with the Asian fauna.

Numerous lineages are shared with the southern half of the continent, most of which are more diversified in the south and display trans-Antarctic ties : Parastacidae, Hyriidae Richardsonianidae, Hydrobiosidae, Triplectidinae, Phreatoicidea ; all, except the latter, are present in New Guinea. Many typically southern lineages of aquatic insects extend along the eastern coast to tropical Australia : Grypopterygidae, Blephariceridae, *Sortosa* (caddisfly), Coenosucidae. Very suggestive is the distribution of the *Oxyethira*-group of ten genera of the prevailingly tropical Hydroptilidae (caddis-flies) (46). Nine of them are present in Australia, three being endemic ; the tenth lives in the geographically close New Zealand and New Caledonia ; only two genera hay a wider distribution from Australia through south and east Asia to Europe. A similar distribution, from Australia to Eurasia, is also displayed by the tribe Apistomyiini of Blephariceridae (48) and by *Ecnomus* (caddisflies (18). All three lineages are not restricted to tropical Australia, being also present in the south and all have Gonwanian ties.

Actually there are also freshwater genera with tropical Asian ties restricted in the Australian region to the tropical north ; they belong to Entomostraca with passive dispersal means (Diaptomidae) or to expansive groups of aquatic insects (*Neoperla*, various caddis flies), i.e. to taxa able to cross marine arms.

Summarizing these data, it results that the primary freshwater animals and aquatic insects from the Australian region (including northern New Guinea) are most closely related to those of other areas formerly in Gondwanaland. The trans-oceanic relations of this fauna involve first of all South America on the one hand. New Zealand/New Caledonia on the other hand, then (south) Africa, Madagascar, even India (Phreatoicidea). The only primary freshwater genera in the north of the region displaying south Asian ties are the archaic *Scleropages* (which may have once been widely ranging) and the probably expansive *Bellamya*.

The fauna of primary freshwater animals and even of aquatic insects of southern Australia obviously is more closely related to that of northern Australia/New Guinea than to the South American one and the northern Australian more colosely related to the south Australian than to the south Asian one. Significant in this respect are the interrelationships of the three genera of the *Tanorus* group (Hydrobiosidae), a lineage shared by the two halves of the Australian region and temperate South America : the south-eastern Australian Megogata shares more characters with the genus from New Guinea (i.e. it is more closely related to it) than with the South American genus (40).

Just the contrary with the faunas of peripheral animals : the north Australian ones consists mainly of Indo-West Pacific lineages also in southern Asian and the southern Australian one of circum-Antarctic lineages shared with cold/temperate South America.

Parenti (33) maps a sharp faunistic limit betwen southern and northern New Guinea. Actually the genuine freshwater fauna of northern New Guinea is an Australian one, like that of southern New Guinea, including members of Melanotaeniidae, hyrid mussels, *Glossamia* (Apogonidae), *Parambassis* (Chandidae), the endemic *Tanorus* (Hydrobiosidae) *Symphitoneurina* (Triplectidinae) which all display sister relations with the Australian/southern New Guinea fauna.

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NUMERICAL STRUCTURE OF COLLEMBOLA POPULATIONS FROM THE SOILS OF RETEZAT NATIONAL PARK (THE CARPATHIANS)

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The paper presents a part of a research carried out during a two years time in three areas located in the Scientific Reservation of the Retezat National Park. All the details regarding material and methods have been presented, Falcă (1972). Numerical density, relative abundance, frequency, constancy and numerical dominancy are presented.

1. INTRODUCTION

Collembola, near Acarina, represents dominant animals in all types of soils, playing an important role in the substance and energy transfer processes taking place in the natural ecosystems.

There are many quantitative surveys concerning the numerical structure of collembola communities. A historical review was made by Murphy (1953), Kevan (1958) and Petersen (1982).

This paper is part of a complex study undertaken not only on the numerical structure of the communities, but also on the biomass and productivity.

2. RESULTS

Numerical density, calculated as the average number of individuals to the square meter, Table 1, showed two group of species : the first one, including the species of the genera Onychiurus, Folsomia, Isotomiella, and Isotoma, presented large numerical densities ; the second one, including the species of the genera Xenilla, Triacanthella, Pseudachorutes, Neanura, Orchesella, and Sminthurus, presented small numerical densities. It was a characteristic feature for all worked areas, having the following vegetal associations ; Festuco (drymeae) — Fagetum (I), Piceetum carpaticum (II), and Pinetum mugi carpaticum (III).

Among the species with large numerical densities, the most representative were *Folsomia quadrioculata* and *Onychiurus armatus*, followed by *Isotoma violacea*, *Folsomia inoculata*, *Isotomiella minor*, *Isotoma notabilis*, and *Onychiurus rectopapillatus*. *Xenilla brevicauda*, *Triacanthella sp.*, *Pseudachorutes dubius*, and *Neanura muscorum* were some of the species with small density.

Standard errors of the means presented high values, especially for the species with small numerical densities and frequency.

Table 1

Numerical density of Collembola species in the Retezat National Park

— Festuco (dymeae) — Fagetum — (I)

Confidence level 95 %+

Species	Level of preleva-tion	1+ Mean 33 sq.cm.	1+ Mean sq.m.	1+ Standard error	1+ Relative abun-dance	1+ Frequency	2+ Mean 33 sq.cm.	2+ Mean sq.m.	2+ Standard error	2+ Relative abun-dance	2+ Frequency
Onychiurus armatus	L	5.2	1585.2	1.7	20.49	46.88	3.9	1065.9	1.8	9.79	54.68
	H	—	—	—	—	67.19	7.1	2067.0	1.3	9.69	70.31
Onychiurus rectopapillatus	L	1.2	387.7	0.7	4.83	17.19	1.2	374.2	0.6	3.46	21.88
	H	—	—	—	—	34.38	1.8	539.0	0.8	2.44	25.00
Folsomia quadrioculata	L	8.7	2584.8	2.9	32.04	70.31	15.5	4572.7	4.4	42.14	78.12
	H	—	—	—	—	81.25	22.4	6738.6	4.0	31.07	95.31
Folsomia inoculata	L	1.6	503.0	0.8	6.22	25.00	6.7	1212.9	3.3	11.19	50.00
	H	—	—	—	—	—	8.7	2581.8	2.2	12.01	65.63
Isotomiella minor	L	1.6	462.3	0.6	5.78	39.38	2.9	831.1	0.9	7.65	53.12
	H	—	—	—	—	—	4.3	1297.7	1.2	5.98	56.25
Isotoma notabilis	L	1.2	254.5	0.8	4.47	17.85	1.1	336.7	0.6	3.06	25.00
	H	—	—	—	—	—	2.1	654.2	0.9	3.08	28.13
Isotoma violacea	L	3.8	971.8	1.7	12.07	33.92	0.9	289.4	0.7	2.67	45.31
	H	—	—	—	—	—	6.7	1970.4	1.9	9.29	53.12
Total (all identified species)	L	27.0	7877.5	12.8			39.6	10892.3	18.6		
	H	—	—	—			—	—	—		

— Piceetum carpaticum — (II)

Species	Level of preleva-tion	1+ Mean 33 sq.cm.	1+ Mean sq.m.	1+ Standard error	1+ Relative abun-dance	1+ Frequency	2+ Mean 33 sq.cm.	2+ Mean sq.m.	2+ Standard error	2+ Relative abun-dance	2+ Frequency
Onychiurus armatus	L	16.0	4903.9	5.7	22.65	64.28	2.8	888.6	1.2	4.71	35.94
	H	—	—	—	—	—	8.2	2459.8	2.2	7.97	54.68
Onychiurus rectopapillatus	L	2.8	799.1	1.7	3.65	17.85	2.1	425.4	1.6	2.31	20.31
	H	—	—	—	—	—	2.8	764.8	1.4	2.35	23.44
Folsomia quadrioculata	L	21.6	6821.2	8.0	28.43	64.28	31.6	9248.8	11.9	49.17	70.31
	H	—	—	—	—	—	32.5	9685.2	7.9	31.80	81.25
Folsomia inoculata	L	2.3	875.3	1.9	3.75	19.64	5.7	1696.9	2.3	8.87	45.31
	H	—	—	—	—	—	12.7	3960.9	3.7	11.70	59.37
Isotomiella minor	L	3.7	1148.9	1.2	5.02	55.35	4.7	1359.8	2.1	7.42	51.56
	H	—	—	—	—	—	7.2	1279.2	2.7	4.29	48.43
Isotoma notabilis	L	1.9	443.3	1.2	2.63	19.64	0.9	287.5	0.5	1.61	18.75
	H	—	—	—	—	—	1.6	417.8	1.1	1.61	15.63
Isotoma violacea	L	9.3	2877.5	2.7	12.26	60.71	1.3	399.2	0.8	2.15	18.75
	H	—	—	—	—	—	6.2	1857.2	2.0	6.18	45.31
Total (all identified species)	L	74.71	22793.6	34.8			64.9	18975.9	33.5		
	H	—	—	—			102.4	31288.6	40.4		

— Pinetum mugi carpaticum — (III)

Species	Level of preleva-tion	1+ Mean 33 sq.cm.	1+ Mean sq.m.	1+ Standard error	1+ Relative abun-dance	1+ Frequency	2+ Mean 33 sq.cm.	2+ Mean sq.m.	2+ Standard error	2+ Relative abun-dance	2+ Frequency
Onychiurus armatus	L	7.2	2113.4	4.9	22.34	71.42	2.3	699.6	1.3	4.44	26.56
	H	—	—	—	—	—	3.1	942.4	1.4	6.63	28.13
Onychiurus sibiricus	L	1.2	354.1	1.0	3.65	19.64	0.8	279.5	0.5	1.61	15.63
	H	—	—	—	—	—	1.8	549.6	1.1	3.87	18.75
Folsomia quadrioculata	L	10.1	3045.4	6.8	31.26	83.92	19.2	5814.8	7.4	37.19	46.87
	H	—	—	—	—	—	15.1	4522.3	5.4	31.82	50.00
Folsomia alpina	L	1.6	474.5	0.9	4.89	25.00	4.4	1350.4	2.3	8.68	35.94
	H	—	—	—	—	—	3.8	634.8	2.7	4.47	28.13

Tabel 1

Species	Level of prelevation	1+ Mean 33 sq. cm.	1+ Mean sq. m.	1+ Standard error	1+ Relative abundance	1+ Frequency	2+ Mean 33 sq. cm.	2+ Mean sq. m.	2+ Standard error	2+ Relative abundance	2+ Frequency
Isotomiella *minor*	L	1.5	427.7	0.9	4.89	21.42	7.8	577.3	2.9	3.67	26.56
	H	—	—	—	—	—	2.7	634.1	1.3	3.17	21.88
Isotoma viridis	L	2.1	643.7	1.9	6.68	14.28	4.9	1724.2	2.9	9.74	26.56
	H	—	—	—	—	—	4.1	1255.3	2.1	8.83	26.56
Isotoma violacea	L	3.6	1137.2	2.2	11.51	21.42	2.4	745.4	1.9	4.83	18.75
	H	—	—	—	—	—	1.3	407.6	1.0	2.87	12.50
Total (all identified species)	L	31.9	9668.9	23.36			63.3	15976.9	34.9		

\+ The tables contain only the first 7 dominant species
1 + The first year of researches
2 + The second year of researches
L = litter
H = humus

The variation coefficient, having small values, made evident species *Folsomia quadrioculata* and *Onychiurus armatus*, with high numerical densities, because of a high biotic potential and ecological plasticity.

Variance showed high values, as compared with those of the means, for the majority of species, which is an indication of the lack of concentration of the numerical density about the mean.

The overdispersion indexes of Bliss and Fischer, with superunity values, indicate grouped distribution of Collembola, according to the negative binomial distribution.

This distribution is characterized by two parameters : the mean and the exponent (k) ; the generative function of probabilities is : $(q - p)^{-k}$, where $p = \frac{m}{k}$; $q - p = 1$; $q = 1 + \frac{m}{k}$.

Folsomia quadrioculata, a dominant species was chosen to put into agreement the experimental distribution with the negative binomial one. By means of the conformity test, we verified the "agreement" quality between the experimental and theoretical data. $x^2 = 6.339$, which corresponds to a probability comprised between 95% and 97.5%, means a very significant "agreement".

Analysing negative binomial distribution, when the variance closes to the mean, the *k* exponent tends to the infinite ; in this case, negative binomial distribution tends to the Poisson distribution . This phenomenon takes place when the populations are small, as *Hypogastrura tullbergi*, *Neanura conjucta*, *Lepidocyrtus lanuginosus*.

From the point of view of total density, the highest values have been presented by the area II with 31288 individuals on a square meter, in humus, followed by the area I, with 21644 individuals on a square meter, and area III, with 15967 individuals on square meter.

The summary characterization of the areas, from the point of view of the density of collembola populations, is a topic problem : do the average densities, in a succession determined by the frequency of observations, refllect the true values of the populations of collembola ? In other words, are the various values of densities, noticed at certain intervals, distinct responses, as a result of the complex processes at the level of these populations ? To point out this response, the null hypotheses were tested by using the differences between the means and the calculations of the levels of significance for species *Folsomia quadrioculata* and *Onychiurus armatus*, dominant species in all areas, as well as the other species taken as a whole. The differences between the means were insignificant in all analysed cases, so the null hypotheses were accepted with the level of significance 5%. It means that the probability of accepting the null hypotheses, is higher than 0.05 supporting the idea that the differences between the means are not explained by other causes, besides the fact that ecological factors influenced the dynamics of numerical densities of the species of collembola.

The relative abundance — table 1 — pointed out the fact that, from the total 65 identified species, a nucleus of 7 species represents 71%. They are the following : *Folsomia quadrioculata*, *Onychiurs armatus*, *Isotoma violacea*, *Isotomiella minor*, *Folsomia inoculata*, *Onychiurus rectopapillatus* and *Isotoma notabilis*. The other 58 species, although ewight times more in

number, participate in variabile percentages, but smaller than those mentioned, to establish the structural configuration of the associations of collembola.

Within the nucleus of 7 species with relative high abundance, the species *Folsomia quadrioculata* and *Onychiurus armatus* represent 34 % and 11 %.

It is to be noticed that in the area III — Pinetum mugi carpaticum — the species *Onychiurus rectopapillatus*, *Folsomia inoculata* and *Isotoma notabilis* were replaced from the nucleus of 7 species by *Onychiurus sibiricus*, *Folsomia alpina* and *Isotoma viridis*.

The frequency — Table 1 — presented similar characteristics to relative abundance with high percentages, of about 80 %, for species *Folsomia quadrioculata* and *Onychiurus armatus* and much lower for species with poor numerical values.

Table 2

Constancy of Collembola — summarising table as species number

Level of collecting	CONSTANT 50 %				ACCESSORIES 25—50 %				ACCIDENTALES 25 %			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
L	2	4	—	6	2	—	2	4	29	31	24	84
H	—	—	—	—	—	—	—	—	—	—	—	—
								2				
L	3	2	—	5	2	4	5	11	44	44	36	124
H	5	3	—	8	4	8	4	16	39	33	31	103

L = litter
H = humus
I — Biotope with vegetal association Festuco (drymeae) — Fagetum
II — Biotope with vegetal asociation Piceetum carpaticum
III — Biotope with vegetal association Pinetum mugi carpaticum

Constancy — Table 2 — the areas with vegetal associations Festuco (drymeae) — Fagetum (I) and Piceetum carpaticum (II), 44 species (84 %) were accidental sepecies, while in the area with the vegetal association Pinetum mugi carpaticum (III) the number of accidental species was of 36, that is 89 %.

Within the category of incidental species, 2, 4 and 5 species were identified, representing therefore 4 %, 8 % and 11 %.

The constant species were few in number, 3 in the area I, 4 in the area II, none in the area III, representing 6 % and 8 % respectively.

Numerical dominance — table 3 — estimated as the average degree of expansion of a species in an interval of time (Da = E/t) pointed out species *Folsomia quadrioculata* and *Onychiurus armatus* with the highest values between 5 and 30, according to the area, both in the litter and humus, in all investigated areas.

Table 3

Numerical dominance of Collembola — summarising table

Level of collecting	Biotope					
	I		II		III	
	1	2	1	1	1	2
L	13.7	21.6	43.2	29.8	16.8	29.8
H	—	45.5	—	65.3	—	27.8

L = litter
H = humus
1 = first year of research
2 = second year of research
x = there are no data drom the first year

I — Biotope with vegetal association Festuco (drymeae) — Fagetum
II — Biotope with vegetal association Piceetum carpaticum
III — Biotope with vegetal association Pinetum mugi carpaticum

Considered as an expression of higher or lower receptivity of habitate to a species or another, the numerical dominance of each species represented, by summing up, the total receptivity of the habitat. From this point of view, it was noticed that the area with the vegetal association Piceetum carpaticum (II) presents the highest value, meaning therefore that here the collembola found the best conditions for their activity — Table 3.

CONCLUSIONS

1. Among the species with high numerical density the most representative from all biotopes were : *Folsomia quadrioculata*, *Onychiurus armatus* followed by *Folsomia inoculata*, *Isotomiella minor*, *Isotoma notabilis*, *Isotoma violacea* and *Onychiurus rectopapillatus* . The overdispersion indexes of Bliss and Fischer, with superunity values, indicated the grouped distribution of Collembola, according to a negative binomial distribution. The biotope with vegetal association Piceetum carpaticum has presented the highest values of numerical density.

2. The relative abundance pointed out that from a number of 65 identified species, a nucleus of 7 species represented 71 % ; *Folsomia quadrioculata* and *Onychiurus armatus* were represented as 34 % and respectively 11 %, having the highest percentages of relative abundance.

3. The frequency of species Collembola was about 80 % for *Folsomia quadrioculata* and *Onychiurus armatus* and had much lower values for the other remaining species.

4. From the point of view of species constancy, 84 % were accidental species in the areas with vegetal associations Festuco (drymeae) — Fagetum and Piceetum carpaticum. Incidental and constant species were much fewer in all studied biotopes.

5. The value of total numerical dominance, that is, the sum of all species numerical dominance, has presented the highest value in the area with vegetal association Piceetum carpaticum. As far as numerical dominance is taken into account as a measure of species receptivity to a spe-

cific habitat, it can be said that Collembola species found better conditions for their activity in the area with the vegetal association Piceetum carpaticum than in the other two studied areas.

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RESEARCHES OF ORIBATID FAUNA (ACARI-ORIBATIDA) IN DIFFERENT TERRESTRIAL ECOSYSTEMS IN THE DANUBE DELTA

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The oribatid fauna in the Danube Delta is little known. The paper comprises the result of systematic, biogeographic and trophical researches of oribatid fauna in different areas of the delta biome, characterized by natural vegetation, consisting in forests and lawns and artificial vegetation of plantations. In these researches, a number of 93 species of oribatids of 31 families was identified.

The Reservation of Danube Delta Biosphere represents a territory of ecological importance both for the national as well as for world interest. The terrestrial ecosystems represented by the delta sand banks with different origins, age and structures have a characteristic flora and fauna that constitute the subject of several researches.

From the soil fauna the oribatid, also known as "horny acariens", that have a most important role in the descomposition processes, populate almost all the terrestrial delta ecosystems. Their presence is conditioned by the multitude of bio-edaphic and climate factors from which the medium relative humidity of air and substratum are the most important ones. The types of soil and vegetal associations, the degree of the soil cover and the thickness of the litter determine the structure and density of the coenosis of the acariens. The previous ecological researches, (10), underlined the quantitative importance of the soil fauna and implicitly the number of individuals of the oribatid species in the functioning of some forestry ecosystems in the Danube Delta.

But a great importance is given to the systematic, biogeographical and trophic framing in the study of populations of these microarthropods.

MATERIAL AND METHOD

The researches developed during 1982–1987 and 1991 in 15 areas placed in different terrestrial ecosystems. The surfaces show the following general characteristics :

1. Letea – Hasmacul Mare – natural of deciduous forest,
2. Letea – Hasmacul Mic – natural of deciduous forest.
3. Cardon – poplar plantation.
4. Cardon – alder plantation.
5. Letea – sand bank in different degrees of vegetations.
6. C.A. Rosetti – halophile lawn.
7. Răducu – lawn.
8. Sviştofca – lawn.
9. Caraorman – lawn.
10. Ivancea – lawn.
11. Sulina – lawn.

12. Sfîntul Gheorghe — poplar plantation.
13. Island Sacalin — halophile lawn.
14. Chituc — halophile lawn.
15. Periboina — halophile lawn.

The natural lawns in the Danube Delta encountered on stretched surfaces are mostly halophilous and quarter a series of rare species from

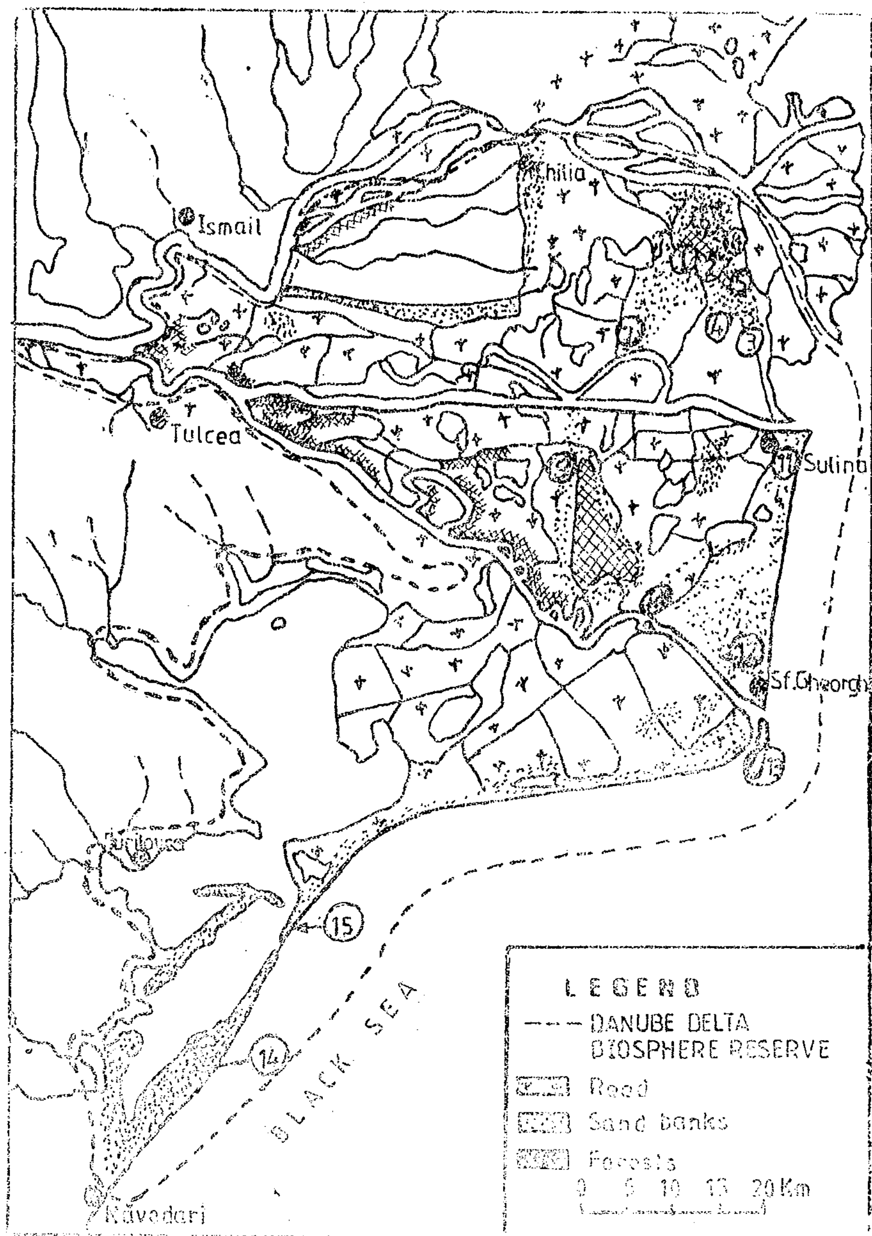

Fig. 1. — Danube Delta — terrestrial ecosystems researched.

the flora and characteristic species of the fauna. The forests previously described present characteristics typical for the forest ecosystems, where we met a typical fauna. In order to study the oribatids in these areas many samples of litter and soil have been collected with a MacFadyen soil core-borer, up to 10 cm deep and have been sorted by the Berlese-Tullgren extractor. The systematic arrangement follows Balogh (1972) criterion, while the biogeographical and trophical distribution is made on the basis of the reference (6, 7, 8, 9) papers. On the list of species and an the map (Fig. 1), the areas are pointed by order number followed by their biogeographical and trophic abbreviations. The biogeographical abbreviations are : Eur. = European ; Cauc. = Caucasian ; Oc. = Oceania ; As. = Asian ; N. — Am. = North-American R. =Romania ; N. — Af. = North-African ; SE.-Eur. = Southeast-European ; C.-S.-Eur. = Central-South-European ; C. -As. = Central-Asian ; s. bip. = subbipolar ; hol. = holarctic ; sbt. = subtropical ; maghreb. = maghrebian ; N.-S.-Am. = North-South-American ; cosmp. = cosmopolitan ; arct. = arctic ; Balc. = Balcanic ; C.-E.-As. = Central-East-Asian ; E.-As. = East-Asian ; C.-Eur. = Central-European ; Sibir. = siberian ; C.-SE.-Eur. = Central-SouthEast-European ; S.-As. = South-Asian ; trop. = tropical ; W.-Sibir. = West-Siberian ; Eurosibir. = Eurosiberian ; C.-W.-Eur. = Central-West-European ; N.-E.-As= North-East-Asian ; pal. = palearctic. The trophical abbreviations are : M. = macrophytophag ; m. = microphytophag ; p. = panphytophag ; no. = nonidentified.

RESULTS AND DISCUSSIONS

The oribatid fauna from the Delta areas registered 93 species belonging to 31 families (see the list of species). Out of the total of families, four are dominant : Phthyracaridae, Opiidae, Oribatulidae, Ceratozetidae, where most of the species, presenting large ecological and biogeographical valences, are included. Out of the number of the species mentioned above, 58 are signaled in the natural and planted forestry ecosystems and 14 for lawns, whereas 21 species are common.

In the natural and planted forest ecosystems we meet a great number of species out of which a large number is characteristic of these types of ecosystems, e.g. : *P. laevigatus*, *P. baloghi*, *P. globosus*, *P. lignaeus*, *P. dubinini*. *A. serratus*, *E. cribrarius*, *E. monodactylus*, *E. intermedius*, *M. pulverulenta*, *G. bicostatus*, *S. subtrigona*, *F. fuscipes*, *P. lanceata*, *Z. exilis*, *Q. quadricarinata*, *C. sexpilosa*. That species appeared near the other species, which we considered as bioindicators for that ecosystems (we mentioned that species in a previous paper).

On the halophilous lawns and sand banks with different degrees of vegetation we meet a small number of species as : *Z. meriehamere*, *Z. tenuelamellata*, *L. theleproctus*, *L. naltschichi*, *S. minutus*, *P. africanus*, which can be considered characteristic for those ecosystems because it is only there they were found. But the smallest number of species are to be found in the halophilous lawns. In those areas there was recorded a typical halophilous species *Z. mariehamere*, which was mentioned by other authors in similar areas (2).

Biogeographically the species mentioned above are dominated by S.-E.-European, Eurosiberian and Asian elements. Most of the European and Eurosiberian species are met in natural and planted ecosystems, the Asian, maghrebian areas in the lawns. Their presence is due to the existence of a calcareous substrate, which created a condition for mesoxerophile and xerophyle species.

From the three trophic groups, the third one, the panphytophagous presents the greatest share (44.08%), followed by the microphytophagous with (15.05%) and macrophytophagous with (11.80%). The group of nonidentified trophic level represents (29.03%), and we can affirm they should be included in the panphytophagous and microphytophagous group, as we found these species in fermentation and hummus layers their specific food exists. That situation is to be met especially in natural and planted ecosystems, and not so much in the lawn ecosystems where the bio-edaphic conditions do not allow the distiuct formation of fermentation and humus layers necessary to the setting of oribatid populations.

CONCLUSIONS

The type of vegetal associations and the bio-edaphic conditions of the Danube Delta divide the oribatid fauna into three groups. : the first one is characteristic for natural and planted forestry ecosystems; the second one is characteristic for halophilous lawns and sand banks and the third one is common for forest areas and the other lawns.

The oribatid fauna of the Danube Delta is original for the presence and interference between European and many Asian elements.

The presence in the terrestrial ecosystems of the Danube Delta of the panphytophaga and of the microphytophaga on a bigger scale justifies an advanced process of decomposition. This process is more intense in the forest ecosystems which, in the case of this special part of Romania, are considered a real natural equilibrium which should not be disturbed as it plays an important part in the functioning of the Delta biome.

LISTS OF SPECIES

PHTHIRACARIDAE Perty, 1841
Phthiracarus Perty, 1841
P. laevigatus (C. L. Koch, 1841) : 1 ; Eur ; M.
P. baloghi (Feider et Suciu, 1957) : 1 ; Eur., Cauc., Oc., As. ; M.
P. globosus (C. L. Koch, 1841) : 1 ; Eur., Or., As. ; M.
P. lignaeus (Willmann, 1931) : 2 ; Eur., Cauc., N. -Am. ; M.
P. dubinini (Feider et Suciu, 1957) : 2 ; R. ; M.
Atropacarus (Atropacarus) Niedbała, 1986
A. serratus (Feider et Suciu, 1957) : 1 ; Eur., N.-Af. ; M.
Euphthiracarus Ewing, 1917
E. cribrarius (Berlese, 1904): 2, 4; Eur.; M.
E. monodactylus (Willmann, 1919) : 1 ; C.-S.-Eur. ; M.

E. intermedius (Feider et Suciu, 1967) : 9 ; SE.-Eur. ; M.
Microtritia Märkel, 1964
M. minima (Berlese, 1904) : 1 ; Eur., Cauc., C.-As., N.-Am. ; M.
Rhysotritia Märkel Meyer, 1959
R. ardua (C. L. Koch, 1841) : 1. 2. 5. ; s. bip.-hol.-sbt. ; no.

ENIOCHTHONIIDAE Grandjean, 1947
Hypochthoniella Berlese, 1910
H. pallidula (C. L. Koch, 1836) : 1. ; hol., sbt. ; no.

EPILOHMANNIIDAE Oudemans, 1923
Epilohmannia Berlese, 1910
E. cylindrica (Berlese, 1904) : 1. ; Eur., maghreb., C.-As., N.-Am., Oc. ; no.

CAMISIIDAE Oudemans, 1900
Camisia von Heyden, 1826
C. biurus (C. L. Koch, 1836) : 3. 6. ; Eur., N.-Am. ; p.

MALACONOTHRIDAE Berlese, 1916
Trimalaconothrus Berlese, 1916
T. tardus (Michael, 1888) : 1. ; Eur. ; no.

LIODIDAE Grangjean, 1954
Liodes von Heyden, 1826
L. theleproctus (Hermann, 1804) : 6. ; Eur., Cauc., Oc., N.-S.,-Am. ; no.

TRHYPOCHTHONIIDAE Willmann, 1931
Trhypochthonius Berlese, 1904
T. tectorum (Berlese, 1896) : 12. ; hol. , cosmop. ; m.

GYMNODAMAEIDAE Grandjean, 1954
Gymnodamaeus Kulczynski, 1902
G. bicostatus (C. L. Koch, 1836) : 2. ; hol., arct. ; m.

DAMAEIDAE Berlese, 1896
Spatiodamaeus Bulanova-Zachvatkina, 1967
S. verticillipes (Nicolet, 1855) : 1. ; Eur. ; m.

BELBIDAE Willmann, 1931
Metabela Grandjean, 1836
M. pulverulenta (C. L. Koch, 1840) : 1. ; Eur. ; m.

DAMAEOLIDAE Grandjean, 1965
Damaeolus Paoly, 1908
D. ornatissimus (Csiszar, 1962) : 1. 2. ; Balc. ; m.
Fosseremus Grandjean, 1954
F. laciniatus (Berlese, 1904) : 1.2. ; Eur., N.-Am. ; m.

ZETORCHESTIDAE Michael, 1898
Zetorchestes Berlese, 1988
Z. micronichus (Berlese, 1883) : 1. 4. 9. ; C.-S.-Eur., maghreb. ; p.
Microzetorchestes Balogh, 1943
M. emeryi (Coggi, 1898) : 3. 4. ; C.-S. Eur. ; p.

LIACARIDAE Sellnick, 1928
Adoristes Hull, 1916
A. ovatus (C. L. Koch, 1840) : 2. ; Eur., N.-Am. ; p.

XENILLIDAE Wooley Higgins, 1966
Xenillus Robineau-Desvoidy, 1839
X. clypeator Robineau -- Desvoidy, 1839 : 2. ; Eur., Cauc., C.-E.-As. no.
X. tageocranus (Hermann, 1804) : 1. ; Eur. maghreb., E.-As. ; p.

ASTEGISTIDAE Balogh, 1961
Cultroribula Berlese, 1908
C. bicultrata Berlese, 1908 : 1. ; C.-Eur., Sibir., N.-Am. ; m.

METRIOPPIIDAE Balogh, 1943
Ceratoppia Berlese, 1908
C. sexpilosa Willmann, 1938 : 4. ; C.-SE.-Eur. ; m.
Pyroppia Hammer, 1955
P. tajikistanica D. Krivolusky et Cristov, 1970 ; 3. ; S.-As. ; no

CARABODIDAE C.L. Koch, 1837
Carabodidae C.L. Koch, 1836
C. minusculus Berlese, 1923 : 1. ; Eur., N-Am. ; M.

TECTOCEPHEIDAE Grandjean, 1954
Tectocepheus Berlese, 1913
T. alatus Berlese, 1913 ; 1.2. 3. 4. 5. 6. ; C.-SE.-Eur ; p.
T. sarekensis (Trägardh, 1910) : 1. ; hol., arct., trop., cosmop. ; p.
T. velatus (Michael, 1880) : 1. 2. 3. 4. 9. 11. 12. ; Eur., N-Am., cosmop. ; p.

OPPIIDAE Grandjean, 1954
Cosmoppia Balogh, 1983
C. ornata (Oudemans, 1900) : 1. 2. 6. 9. 7. ; Eur., N.-Am. ; p.
Ctenopiiella Gordeeza Karppinean, 1988
C. obsoleta (Paoli, 1908) : 1. 2. 3. 4. 5. ; Eur., W.-Sibir. ; p.
Hypogeoppia Subias, 1982
H. sigma var conjuncta (Strenzke, 1951) : 7. ; C.-Eur., C.-As. ; p.
Microppia Balogh, 1983
M. minus (Paoli, 1908) : 1. 2. 3. 4. 6. ; hol. ; p.
M. minutissima (Sellnick, 1950) : 1. ; C.-SE..Eur. ; p.
Multioppia Hammer, 1961
M. laniseta Moritz, 1966 : 1. 2. 3. 4. ; Eur. ; p.
Oppiella Jacot, 1937
O. falcata (Paoli, 1908) : 1. 7. 9. 10. ; Eur., Cauc. ; p.
O. neerlandica (Oudemans, 1900) : 1. 2. 4. 3. 5. 6. ; Eurosibir. ; p.
Quadroppia Jacot, 1939
Q. quadricarinata (Michael, 1885) : 4. ; hol., sbt., ; p.
Ramusella (Insculptoppia) Subias, 1980
R. clavipectinata (Michael, 1885) : 4. 12. ; Eur. ; p.
R. insculpta (Paoli, 1908) : 1. 2. 3. 4. ; Eur. ; p.

Suctobebab Paoli, 1908
S. trigona (Michael, L888) : 2. 6. ; Eurosibir., Eur., Cauc., W.-As. ; p.

Suctobelbella Jacot, 1937
S. acutidens (Forsslund, 1941) : 1. 2. 3. 4. ; Eur., N.-Am. ; m.
S. subcornigera (Forsslund, 1941) : 1. 2. 3. 4. 9. 12. ; Eur., N.-Am. ; p.
S. subtrigona (Oudemans, 1980) : 1. ; Eur. ; p.
S. baloghi Forsslund, 1958 : 1. 2. 3. 4. ; Eur. ; p.

MICREREMIDAE Grandjean, 1954
Micreremus Berlese, 1908
M. brevis (Michael, 1888) : 1. ; Eur., W.-Sibir., C.-As. ; m.
Passalozetes Grandjean, 1932
P. africanus Grandjean, 1932 : 5. 15. ; Eur., C.-As., maghreb. ; no.
P bidactylus (Coggi, 1900) : 14. ; Eur., C.-As. ; no.

SCUTOVERTICIDAE Grandjean, 1954
Scutovertex Michael, 1879
S. minutus (C. L. Koch, 1936) : 5. ; Eur., maghreb. ; m.
S. sculptus (Michael, 1879) : 12. ; C.-W.-Eur., ; maghreb. ; m.

ORIBATULIDAE Thor, 1929
Oribatula Berlese, 1896
O. tibialis (Nicolet, 1855) : 12. ; hol. ; m.
Scheloribates Berlese, 1908
S. labyrinthicus Jeleva, 1962 : 2. 12. ; S.-E.-Eur. ; p.
S. laevigatus (C. L. Koch, 1836) : 2. 8. 9. ; Eur., maghreb., N.- Am. trop. ; p.

S. latipes (C. L. Koch, 1844) ; : 1. 4. ; S.-E.-Eur. ; no.
S. pallidulus (C. L. Koch, 1840) : 1. 2. 12. ; Eur., N.-S.-Am. ; p.
S. distinctus Mihelcic, 1964 : 1. 3. ; S. -Eur. ; no.
Zygoribatula Berlese, 1917
Z. exilis (Nicolet, 1855) : 4. ; Eur. ; m.
Z. cognata (Oudemans, 1902) : 4. ; C.-S.-Eur. , no.
Z. tenuelamellata Mihelcic, 1956 : 6. ; Eur. ; no.
Z. meriehamere Feider, Vasiliu și Magda Călugăr, 1970 : 6. 13. 15. ; R. ; m.

HAPLOZETIDAE Grandjean, 1936
Haplozetes Willmann, 1935
H. vindobonensis Willmann, 1935 : 2. 3. 4. ; Eur., maghreb. ; no.
Protoribates Berlase, 1908
P. monodactylus (Haller, 1884) : 1. 2. 3. 4. 8. 9. 10. ; hol. ; p.

CHAMOBATIDAE Grandjean, 1954
Chamobates Hull, 1916
C. cuspidatiformis (Trägardh, 1904) : 7. 9. ; Eur. ; p.
C. cuspidatus (Michael, 1884) : 1. 2. 4. 7. 9. ; Eur., N.-Am. ; p.
C. spinosus Sellnick, 1928 : 1. 2. 4. 7. 9. ; Eur. ; p.

EUZETIDAE Grandjean, 1954
Euzetes Berlese, 1908
E. globulus (Nicolet, 1855) : 1. ; Eur., maghreb. ; p.

CERATOZETIDAE Jacot, 1925

Ceratozetella Shaldybina, 1966

C. sellnicki (Rajski, 1958) : 2. 3. ; C.-SE.-Eur., W.-Sibir. ; no

Ceratozetes Berlese, 1908

C. gracilis (Michael, 1884) : 1. ; cosmop., trop., sbt., s. bip. ; p.

C. fusiger Mihelčič, 1956 : 2. 5. ; Eur. ; no.

Fuscozetes Sellnick, 1929

F. fuscipes (C. L. Koch, 1844) : 3. ; Eur. ; p.

Globozetes Sellnick, 1928.

G. tricuspidatus (Willmann, 1953) : 1. ; C.-E.-Eur. ; no.

C. longipilus (Sellenick, 1928) : 2. ; C.-S.-Eur. ; no.

Latilamellobates Shaldybina, 1971

L. naltschichi (Shaldybina, 1971) : 5. ; Eurosibir. ; no.

Trichoribotes Berlese, 1910

T. trimaculatus (C. L. Koch, 1836) : 3. 9. ; Eur., N.-Am. ; p.

MYCOBATIDAE Grandjean, 1954

Minuthozetes Hull, 1916

M. pseudofusiger (Schweizer, 1922) ; : 2. ; Eurosibir. ; no.

Mycobates Hull, 1916

M. carli (Schweizer, 1922) : 2. ; Eurosibir. no.

Punctoribates Berlese, 1908

P. punctum (C. L. Koch, 1839) : 1. ; hol. ; p.

PELOPIDAE Ewing, 1917

Eupepops Ewing, 1917

E. acromios (Hermann, 1804) : 7. ; Eur., maghreb., W.-Sibir., E.-As. ; p.

Peloptulus Berlese, 1908

P. phaenotus (C. L. Koch, 1844) : 1. 3. 12. ; Eur., W.-Sibir., E.-As. ; p.

ORIBATELLIDAE Jacot, 1925

Oribatella Banks, 1895

O. berlesei Michael, 1898 : 1. 2. 5. ; Eur., Cauc. ; no.

O. calcarata (C. L. Koch, 1836) ; 2. 7. 9. 12. ; Eur., Sibir., N.-Am. p.

O. dudichi Willmann, 1938 : 1. 2. ; C.-SE.-Eur. ; no.

O. meridionalis Berlese, 1908 : 1. 2. ; C.-S.-Eur., N.-E.-As. ; no.

ACHIPTERIIDAE Thor, 1929

Achipteria Berlese, 1885

A. coleoptrata Linné, 1746 : 1. 3. ; hol. ; M.

A. nitens (Nicolet, 1855) : 1. 3. 4. 9. ; pal. ; p

GALUMNIDAE Jacot, 1925

Galumna von Heyden, 1826

G. elimata (C. L. Koch, 1841) : 1. 3. ; Eur., cosmop., trop. ; p.

G. lanceate Oudemans, 1900 : 12. ; Eurosibir. ; p.

Pilogalumna Grandjean, 1956

P. allifera (Oudemans, 1919) : 2. 3. 4. 6. ; C.-S.-Eur. ; p.

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EFFECT OF MAGNESIUM-GLUTAMOGLUCONATE ON THE BASAL AND INSULIN-DEPENDENT DIAPHRAGMATIC GLUCOSE UPTAKE IN STRESSED YOUNG WISTAR RATS

J. MADAR, NINA ŞILDAN and G. FRECUŞ

In 30–, 45– and 60-day-old male albino Wistar rats formalin stress was induced by injecting s.c. daily doses of 0.25 ml of 2 % formalin solution per 100 g b.w., for 5 days. Under this condition an age-related modification of "in vitro" basal diaphragmatic glucose uptake as well as an age-dependent diaphragmatic insulin resistance were observed. The treatment of animals with Mg-glutamogluconate (10 mg per day per 100 g b.w., administered i.p. from 5 % aqueous solution, for 5 days) on the basis of stress-induction, counteracted the insulin resistance of diaphragms, normalising their insulin-promoted net glucose uptake in an age-related manner. The conclusion is drawn that the age of young rats is a major conditioning factor both in stress-evoked insulin resistance of diaphragms and in the ameliorative "antistress" and "proinsulinic" actions of the treatment with Mg-glutamogluconate upon stress-elicited muscular insulin resistance.

It is well established that magnesium has a multiple role in the regulation of carbohydrate metabolism homeostasis in mammals by interactions with the principal glucoregulatory hormones, being necessary for insulin biosynthesis and deposition of catecholamines (2), (3), influencing at the same time, directly or indirectly, the activity of beta-adrenergic receptors for adrenaline (16), (17), (18), and regulating the intramolecular translocation of phosphate-group from glucose-6-phosphate to glucose-1-phosphate (19).

As we reported elsewhere, the stress-induced age-related hyperglycemia in white rats is strongly linked to the muscular insulin resistance, i.e. to the reduction of insulin stimulated glucose utilisation at the level of striated muscles (7), (8), (11), (12), (13).

Starting from the above establishments, as wel as from the ameliorative effects of the treatment with Mg-glutamogluconate upon the age-related stress-evoked hyperglycemia in young rats (9), (10), in the present study we investigated the rate of "in vitro" glucose uptake by the isolated diaphragmatic muscles, depending on the age of young rats, under basal conditions, under formalin stress-stimulus, as well as under formalin stress-induction associated with Mg-glutamogluconate administration.

MATERIALS AND METHODS

For experiments 30-, 45- and 60-day-old male albino Wistar rats from the stockfarm of our laboratory were used. The animals were kept under standard laboratory and dietetic conditions. Each age-series of animals was divided into three groups, as follows :

I. Untreated normal groups ; II. Stressed groups by formalin administration ; and III. Stressed groups by formalin administration and treated on this basis with Mg-glutamogluconate.

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Before sacrification of individuals, the daily stress-stimulus, for a period of 5 days, was produced by subcutaneous injection of 0.25 ml formalin solution of 2% ("Chemapol", Czechoslovakia) per 100 g. b.w. into the interscapular region of the skin. At the same time, the controls were injected with 0.25 ml of 0.9% NaCl solution. Simultaneously with stress-induction, Mg-glutamogluconate (prepared and purchased by "Terapia" Cluj-Napoca, Romania) was injected intraperitoneally in daily doses of 10 mg per 100 g b.w., for 5 days, using 0.20 ml from 5% freshly prepared aqueous solution of this compound. The control normal animals and without Mg-treated stressed ones were injected i.p. with 0.20 ml sterilized distilled water per 100 g b.w.

After a fasting period of 16 — 18 hours, and 24 hours following the above interventions, all the animals were sacrificed by cervical dislocation, decapitation and exsanguination.

The diaphragms were quickly excised and immersed for 20 minutes in ice-cooled (+ 4°C) Krebs-Henseleit bicarbonate solution (pH = 7.4), without glucose, and divided into approximately equal halves. From each animal a hemidiaphragm was used for testing the basal "in vitro" glucose uptake, while on the other hemiorgan the insulin-stimulated glucose uptake was tested. The incubation medium was 1.0 ml Krebs-Henseleit bicarbonate solution (pH = 7.4), containing 16.7 micromoles glucose (p.a. "Merck") and 2 mg calf-skin gelatine (p.a. "Merck"). Recrystallized glucagon-free ox-insulin ("Calbiochem", California, potency, 23 I.U. per mg, gerade B) was used in a final concentration of 10^{-3} I.U. per ml incubation medium.

The incubation of hemiorgans was performed for 2 hours at 37.6°C in an original device (6), with a gas phase of 95% O_2 + 5% CO_2 and a shaking velocity of 90 oscillations per minute and 5 cm amplitude, according to our procedure (7), (8).

The initial and final glucose concentration of the incubation medium was determined enzymatically using a Test-Combination GOD-Perid Kit ("Boehringer", GmbH, Mannheim, Germany), according to Werner *et al.* (23). The colour intensity of the samples and of glucose standard was measured spectrophotocolorimetrically at 610 nm, using a "Specol" apparatus (Carl Zeiss, Jena, Germany).

The rate of basal glucose uptake (BAS) as well as the rate of global glucose uptake in the presence of insulin (INS) by the hemidiaphragms were calculated in micromoles per 100 mg fresh tissue per 2 hours. The insulin-sensitivity of diaphragmatic muscle was evaluated by calculating the insulin-stimulated net glucose uptake, i.e. by estimating the values of Δ(INS-BAS).

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

RESULTS

As one can see from the data summarized in Table 1 and Figure 1, under normal conditions the rate of basal diaphragmatic glucose uptake (BAS) in age-groups of 30-, 45- and 60-day-old animals is equal to

4.59±0.25, 4.06±0.30 and 3.46±0.20 micromoles per 100 mg fresh tissue per 2 hours, respectively. Under stress condition, in the case of 30- and 60-day-old animals the rate of diphragmatic basal glucose uptake vs. the

Table 1

Basal glucose uptake (BAS), global glucose uptake in the presence of insulin (INS) and insulin-stimulated net glucose uptake (ΔINS-BAS) by isolated hemidiaphragms from normal (N), stressed (S) and with magnesium glutamogluconate treated stressed (SMgGG) male albino young Wistar rats of various ages

Groups	micromole glucose uptake per 100 mg tissue for 2 hrs		
	BAS	INS	Δ INS—BAS
30-day-old-animals			
N	4.59±0.25(8)	6.96±0.32(8)	2.37±0.18(8)
S	4.32±0.20(8) −5.88%, P> 0.50[a)]	4.89±0.18(8) −29.78%, P< 0.001[a)]	0.56±0.05(8) −76.37%, P<0.001[a)]
SMgGG	4.29±0.18(8) −6.53%, P>0.10[a)] −3.00%, P>0.50[b)]	6.40±0.33(8) −8.04%, P>0.10[a)] +30.87%, P<0.01[b)]	2.11±0.16(8) −10.97%, P>0,25[a)] +276.78%, P<0.001[b)]
45-day-old-animals			
N	4.06±0.30(8)	6.27±0.26(8)	2.21±0.23(8)
S	3.18±0.25(8) −21.61%, P<0.05[a)]	4.45±0.18(8) −29.10%, P<0.001[a)]	1.27±0.24(8) −42.84%, P = 0,01[a)]
SMgGG	4.15±0.26(8) −2.19%, P>0.50[a)] +30.36%, P<0,001[b)]	6.56±0.28(8) +4.62%, P> 0.25[a)] +47.58%, P<0.001[b)]	2.42±0.26(8) +9.07%, P>0.50[a)] +90.84%, P<0.001[b)]
60-day-old animals			
N	3.46±0.20(8)	4.91±0.21(8)	1.45±0.15(8)
S	3.03±0.16(8) −12.42%, P>0.10[a)]	4.04±0.22(8) −17.72%, P = 0.01[a)]	1.01±0.11(8) −30.43%, P<0.05[a)]
SMgGG	3.08±0.22(8) −10.48%, P>0.25[a)] +1.65%, P>0.50[b)]	4.43±0.33(8) −9.77%, P>0.25[a)] −6.95%, P>0.05[b)]	1.35±0.16(8) −10.00%, P>0.50[a)] +33.66%, P<0.05[b)]

(The values represent means ±S.E. The number of experiments is given in brackets.
[a)] Percent modifications and P vs the corresponding N groups; [b)] Percent modifications and P vs. S groups).

corresponding control values is not appreciably changed (— 5.88%, P>0.50 ; and —12.42%, P>0.10, respectively), while in animals of 45-day age this phenomenon is significantly reduced (—21.61%, P<0.05). The treatment of animals with Mg-glutamogluconate on the basis of formalin-

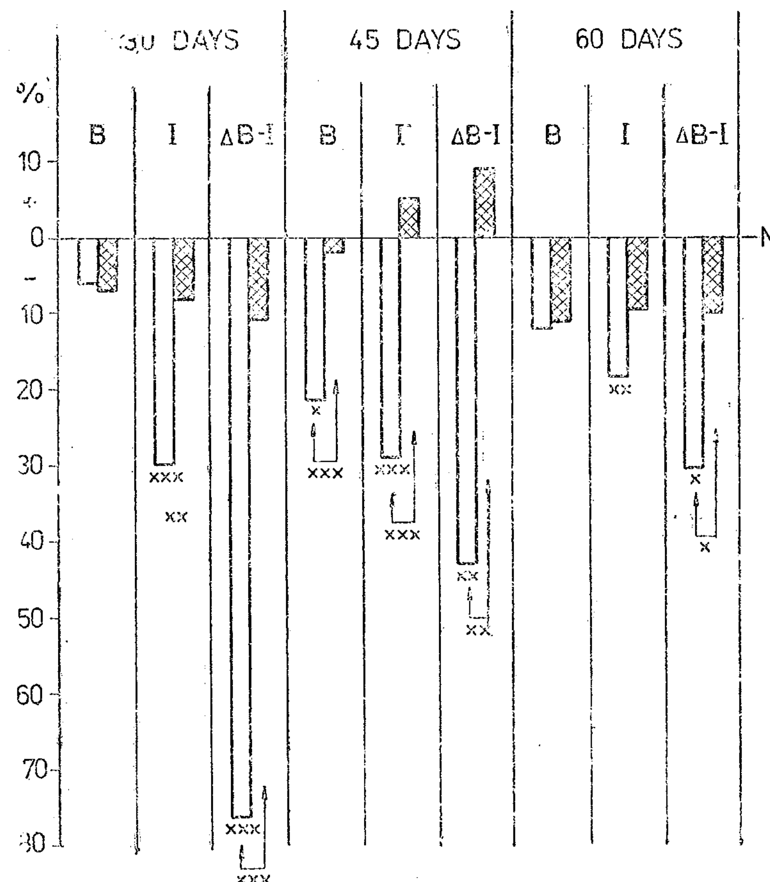

Fig. 1. — Percent differences vs. the corresponding normal values (N) of "in vitro" basal glucose uptake (B), global glucose uptake in the presence of insulin (I) and of insulin-promoted net glucose uptake (ΔI—B) by the hemidiaphragms isolated from 30—, 45— and 60-day-old Wistar rats. (White columns — stressed groups; shaded columns = with magnesium glutamogluconate treated stressed groups; *)P < 0.05; **)P < 0.01; ***) P<0.001. The arrows indicate significant modifications between stressed and magnesium-treated stressed groups).

induced stress, in 30-day-old animals does not influence appreciably the rate of basal diaphragmatic glucose uptake nor vs. the corresponding normal value, or vs. that obtained under stress condition (—6.53%, P> 0.10 and —3.00% P> 0.50, respectively). On the contrary, this treatment in the case of 45-and 60-day-old stressed rats counteracts the inhibitory effect of stress situation upon the rate of basal diaphragmatic glucose uptake, normalizing this phenomenon.

The presence of exogenous insulin (10^{-3} I.U. per ml) in the incubation medium, in all three age-series of rats, stimulates the glucose penetration rate into the muscle pieces, depending on the experimental conditions, as compared to the corresponding basal values and expressed in insulin-stimulated global diaphragmatic glucose uptake (INS).

As referring the insulin-stimulated net-glucose uptake by the diaphragms, calculated by Δ INS-BAZ, it is obvious that under normal conditions this phenomenon is equal to 2.37 ± 0.18, 2.21 ±0.23 and 1.45 ± 0.15 micromoles per 100 mg diaphragm for 2 hours, in 30-, 45- and 60-day-old animals, respectively. Under stress condition, this parameter vs. the corresponding normal diaphragmatic insulinsensitivity is markedly reduced, being diminuished by 76.37% in 30-day-old rats (P<0.001), by 42.84% in 45-day-old animals (P = 0.01), and by 30.34% in the 60-day-old ones (P<0.05), respectively. The treatment with Mg-glutamogluconate of stressed rats, in all three age-series of rats, strongly reduces the stress-evoked diaphragmatic insulin resistance, stimulating the insulin-dependent rate of net glucose uptake by tissue pieces with 276.78% in 30-day-old animals (P<0.001), with 90.84% in 45-day-old rats (P<0.001) and with 33.66% in 60—day-old animals (P<0.05), respectively, as compared to the coresponding values obtained under stress condition, normalizing at the same time the insulinresponsiveness of stressed diaphragms.

DISCUSSIONS

Since the classical isolated rat-diaphragm method for the study of muscular carbohydrate metabolism was elaborated (4), (5), (6), (19), (21), hemidiaphragms from white rats are largely utilized in various experimental models for testing the glucose uptake, insulin sensitivity and insulin resistance of striated muscles, considered as major blood glucose consumers, in this species (6), (7), (8), (22).

From the data presented in this study it is evident that under the influence of acute formalin stress-stimulus, in 30-, 45- and 60-day-old Wistar rats the basal diaphragmatic glucose uptake decreases only in the case of 45-day-old animals, while the insulin-promoted net glucose utilization shows an inverse proportional diminution with advancing in age of individuals. These observations are in good agreement with our earlier findings that the age of Wistar rats greatly influences the diabetogen-hyperglycemic and antiinsulin actions of stress situation (9), (11), (12), (13).

Regarding the age-related ameliorative effect of Mg-glutamogluconate administration upon the stress-evoked diaphragmatic insulin resistance, it is pertinent to assume that in this mechanism the attenuation of the effects of stress-induced adrenaline and corticosterone excess is mainly involved. In fact, our recent data demonstrate that the administration of propranolol (a beta-adrenoceptor blocking compound) on the basis of formalin stress-induction significantly reduces the diaphragmatic insulin resistance (8), hyperglycemia, thymolysis and adrenal hypertrophy, depending on the age of young Wistar rats (9), (12), (11), (13). On the other hand, from the recent observations in our laboratory it is obvious that in athero-

genic stress, Mg-glutamogluconate reduces the concentration of circulating free fatty acids (19), which are preferentially utilized as energetic substrates vs. the circulating glucose at the level of striated mucles (15) as a consequence of adrenaline and glucocorticoid excess (8), (10), (16), (17), (18). Furthermore, it has been established that in the central nervous system (CNS) of rats exist higly Mg-sensitive receptors and therefore Mg-glutamogluconate exerts a protective role in the stress reactivity of CNS against the excitatory effects of xenobiotic agents (1), attenuating at the same time the reactivity, of hypothalamic-hypophyseal-adrenal axis, reducing the secretion of CRF, ACTH, corticosterone and adrenaline, and consequently diminishing the adrenal hypertrophy and tyhmolysis in response to stress stimulus (14).

Recent data show that Mg stimulates insulin biosynthesis and is necessary for adrenaline deposition (2), (3), thus participating in the maintenance of carbohydrate metabolism homeostasis. On the basis of some recent works it is concluded that the stress caused by strong physical exercise is capable of inducing Mg deficit through various mechanisms (16), (17), (18). A possible explanation for decreased plasma Mg concentration during physical stress is the beta-adrenergic lipolysis (16), (17), (18), since fatty acids are nobilized for muscle energy instead of glocose, lipolysis causing a decrease in plasma Mg, by chelating the circulating Mg with free fatty acids and by captation of Mg ions by the adrenaline-activated adipocyte membranes (16), (17), (18).

The mention must be made that the ameliorative action of Mg-glutamogluconate upon the stress-induced age-dependent diaphragmatic insulin resistance, under our experimental conditions, is strongly correlable with its antistress action on stress-evoked hyperglycemia (11), (12). This fact suggests the possibility that magnesium-glutamogluconate is involved in the improvement of insulin sensitivity at the level of striated muscles. Our older data (20) show that $MgCl_2$ added to the incubation medium of isolated rat-hemidiaphragms significantly stimulates the effect of exogenous insulin upon the rate of glucose uptake. On this basis we consider that such a direct action, beside the indirect mechanisms described above, of Mg-glutamogluconate on insulin-dependent transsarcolemmal glucose transport may occur also in the case of stressed rat-diaphragms. For testing this supposition, the investigation of the possible direct effect of Mg-glutamogluconate upon the stress-induced diaphragmatic insulin resistance is in progress.

CONCLUSION

The presented data suggest the conclusion that the age of young Wistar rats is a major conditioning factor both in stress-evoked muscular insulin resistance and in the ameliorative "antistress" and proinsulinic actions of the treatment with Mg-glutamogluconate upon the stress-evoked muscular insulin resistance.

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METABOLIC MODIFICATIONS INDUCED BY TIMOROM® TREATMENT IN MALE AND FEMALE WISTAR RATS

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Adult male and female Wistar rats were treated with Timorom® for 45 days, in a daily dose of 5.0 mg/kg body weight. At the end of the experiment biochemical modifications were different in male and female rats. Glycemia, liver glucose-6-phosphatase (G6Pase) and glycogen phosphorylase *a* (GPa) activities were decreased and liver glycogen storage was increased in the male rats. On the other hand in female group, glycemia, liver G6Pase and GPa activities were increased, while liver glycogen storage was decreased. In our opinion some of these different biochemical modifications obtained in male and female rats by Timorom® treatment depend on the different way of glycogen phosphorylase system activation.

The Timorom® is a drug used in glaucoma treatment. It contains timolol, a nonselective beta blocking agent (4,7).

Because it is well known that beta-blocking agents, widely used in medical therapeutics, have some secondary negative effects such as an increase of serum light density lipoprotein/ high density lipoprotein ratio (4), our purpose was to observe a posssible secondary effect of Timorom® treatment on some of carbohydrate metabolic parameters in rats.

MATERIALS AND METHODS

Our experiments were performed on adult male and female Wistar rats.

The following groups (of 8 animals each) of both sexes were set : male control group (Cm) ; female control group (Cf) ; male group which received Timorom® in the food (5.0 mg/ kg b.w., daily, for 45 days) (Tm) ; female group which received Timorom® in the food as group Tm (Tf).

At the end of the experiment, the animals were killed by decapitation at 9 o'clock in the morning, after a previous fasting for 16 hours. The liver was immediately sampled and the following determinations were performed : glycogen content (11), glycogen phosphorylase *a* (GPa) (6) and glucose-6-phosphatase (G6Pase) (5) activities. We performed the glycemia (13) as well.

The results obtained were processed statistically using Student's "t" test (17). The homogeneity of the media was tested using Chauvenet's criteria and the aberrant values were eliminated. The statistical significance was considered at $p = 0.05$.

RESULTS AND DISCUSSION

Table 1 gives the mean values and the standard error for the controls and for the group which were receiving Timorom®.

The treatment with Timorom® for 42 days caused important modifications of the studied metabolic parameters. It is noticeable that the

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sense of those modifications were sex dependent: glycemia lowered in the male rats (−12.05%) and was increased in the female rats (+47.35%); liver hlycogen storage was elevated in the male rats (+292.00% and decreased in the female rats (−29.89%); G6Pase activity decreased in the male

Table 1

The effect of Timorom R administration on glycemia (g), liver glycogen content (G), liver glycogen phosphorylase *a* (GPa) and glucose-6-phosphatase (G6Pase) activities in male and female Wistar rats

Groups	g (mg%)	G (mg/g fresh tissue	G6Pase (nmols Pi liberated/minute/ mg protein)	GPa (nmols Pi liberated/minute/ mg protein)
Cm $\bar{X} \pm SE$	107.9±1.9	13.26±1.23	11.67±0.31	24.92±1.23
n	8	8	7	8
Tm $\bar{X} \pm SE$	94.9±3.3	51.98±1.65	10.37±0.39	10.38±1.00
n	7	8	8	8
p <	0.01	0.001	0.05	0.001
±Cm	−12.05	+292.00	−11.13	−58.43
Cf $\bar{X} \pm SE$	67.8±2.2	16.19±1.29	8.64±0.72	18.55±0.99
n	8	7	8	8
Tf $\bar{X} \pm SE$	99.9±3.1	11.35±1.38	13.24±0.81	23.86±1.28
n	8	8	8	7
p <	0.001	0.05	0.001	0.01
±Cf%	+47.35	−29.89	+54.40	+28.52

Note: $\bar{X}$ = means; ±SE = standard errors; n = number of individual values; p = signification limit; ±Cm% and ±Cf% = percentage differences versus controls.

(−11.13%) and increased in the female animals (+54.40%); finally, GPa activity was decreased in the male rats (−58.43%) and increased in the female ones (+28.52%).

The glycogenolytic action of beta adrenergic agents in the liver appears to be mediated via the elevation of cellular levels of cyclic AMP (18). All the enzymatic steps of this process have demonstrated, i.e., the activation of adenylate cyclase (14), cyclic AMP-dependent protein kinase (2), phosphorylase kinase (16), and phosphorylase (18), More recently, the glycogenolytic action of epinephrine (8, 15) has been demonstrated to be also mediated by alpha-adrenergic receptors, but this process is not accompanied by an elevation of cyclic AMP levels (2, 9, 19).

It comes out that hepatocytes of juvenile male rats exhibit both alpha- and beta-adrenergic receptors activation of glycogen phosphorylase, where as in older rats the alpha-adrenergic response becomes predominant (1, 12, 19). McMillian et al. (10) found that the number of beta-adrenergic receptors decreased slowly during the development (up to 100 days), while the number of $alpha_1$- receptors increased from birth to the 26-th postnatal day.

Our animals aged 75 days, an age when beta-adrenergic route in glycogen phosphorylase system activation is still operative. We found that treatment with Timorom® caused in male rats a decrease of liver G6Pase and GPa activities; enzymes implied in the glycogen breakdown. It is possible that GPa inactivation was due to a beta-adrenergic blockade induced by Timorom®, on the one hand, a dificiency of the catecholamine action via alpha-adrenergic receptors, on the other hand. In our opinion the increase of liver glycogen storage in male rats may be due concomitantly to an activation of the glycogen synthase system. There were reported in the literature some differences between males and females regarding both alpha-and beta-adrenergic ways to implement liver glycogenolysis; alpha-adrenergic route being more important in adult female rats (Studer & Borle, cited by 3). We found in female rats an accentuated glycogenolysis which was correlated with an increase in the liver GPa and G6Pase activities, and with glycemia too, after Timorom® treatment (see Table 1). In our opinion GPa activation and then liver glycogen breakdown in female rats, were made rather by Ca^{++} than by cyclic AMP as second messenger, because consecutive to the beta-adrenergic blockade, alpha adrenergic route (a cAMP-independent pathway), very important in adult female rats, has become the single modality to activate the phosphorylase system.

CONCLUSIONS

1. A 45 days Timorom® treatment caused modifications in carbohydrate metabolism of both male and female Wistar rats.
2. The sense of those modifications was sex dependent.

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HEPATIC HEMATOPOIESIS EXPERIMENTALLY INDUCED IN THE ADULT MOUSE

PAULA PRUNESCU

Chronic anaemia experimentally induced by phenylhydrazine led to the appearance of all types of hematopoietic colonies in the liver of the adult mouse. Erythroid and blast colonies were the most frequent, but the other types like granulocytic, megakaryocytic and mixed colonies were also present. Ultrastructural features of different types of hematopoietic colonies occurring in the territory of the adult liver were presented.

In the adult mammals, the only source of blood cells is the red bone marrow. Foci of extramedullary hematopoiesis which may appear in man after birth are always associated with a series of diseases, such as decompensated anaemias, infections diseases, idiopathic myelofibrosis and myelosclerosis, cancers (2).

Experimentally, the extramedullary hematopoiesis process in adult mammals can be modelled by drastic conditions of anaemia induction. In the recent literature there are only few papers dealing with the extramedullary hematopoiesis. Nevertheless, various experimental methods such as : bleeding, hypoxia, phenylhydrazine, erythropoietin, hypertransfusion, endotoxin, methylcellulose (4), (13), (14), (20), aimed at the obtaining of hepatic hematopoiesis.

Using the method of phenylhydrazine induction of a decompensated anaemia (17), (23), we obtained the hematopoietic colonies in the liver of the adult mouse and studied them by electronmicroscopy.

MATERIAL AND METHOD

Adult male albino mice weighing 25—28 g were distributed into groups of 8 individuals each. A different number of intraperitonelly phenylhydrazine inoculations performed at intervals of 4 days werre carried out in every group, respectively, 3, 7, 14 and 20 inoculations. The animals were sacrificed 24, 48 and 72 hours after the last inoculation. Many animals, especially in the last inoculation group, died in the course of the experiment. After 7 inoculations a few animals were held for a month without any treatment, after which they were submitted to 14 inoculations. The survivors were sacrificed 48 hours after the last inoculation. The administered doses ranged from 30 to 100 mg phenylhydrazine hydrochloride/ kg body weight of living animal (19), (25). The solution was prepared extemporaneously. The animals were sacrificed by decapitation. For the red blood cells count, blood was sampled on EDTA-Na (21). Liver fragments were fixed in 10% salt forma line and processed according to the routine histologic techniques. The parafine tissue sections thick of 4—5 μ were stained with hemalum eosine and Perls reaction for the detection of ferric iron (11). For the identification of the hematopoietic colony types the Giemsa-colophony staining

was used (15). The hematopoietic colonies counted on sections were performed according to the technique described by Pfrimmer (13). To compare the anaemia levels, the control values of the red blood cells/mm^3 were taken after Russel and Bernstein (16).

For ultrastructure research, small liver fragments were fixed in 2.5% glutaraldehyde solution in cacodylate buffer at a pH 7.2 for 12 h, and postfixed in the same buffered 1,33% osmium tetraoxide solution, for 2 h at the room temperature and in the darkness. The material was included in a Vestopal type medium produced in Romania. The ultrathin sections were stained with uranyl acetate and contrasted with lead citrate . Observations were performed on JEM 7 Electron Microscope at 60 MeV.

RESULTS

In the blood of all experimental groups a marked lowering of the number of erythrocytes/mm^3 was recorded (Table 1).

In the liver, Kupffer cells were the first which reacted to the hemolytic treatment. They were activated for the clearing activity of the lysed erythrocytes in the blood stream. They were marked by loading with siderin and ferritin and appeared strongly Perls-positive. The sinusoids were dilated and loaded with mononuclear cells, among which erythroblasts were identified.

Tabel 1

Development of Anemia and Hepatic Hematopoiesis during the Phenylhydrazine Treatment

Treatment		Red cells 10^6 mm^3	Hepatic Hematopoiesis Colonies ($X \pm SE$/200 microscopic fields)					
Nr. inoc. PH	Time of sacrif.		TOTAL	E	G	M	Mx	B
3	24 h	6.02	232±12	72±2	21±6	28±6	27±4	83±4
	48 h	5.22	567±57	198±1	79±24	28±6	24±3	238±58
7	24 h	4.55	188±20	66±13	39±3	9±3	8±2	64±12
	48 h	4.22	470±68	135±40	120±3	34±19	15±8	163±32
14	24 h	2.40	204±26	50±7	57±12	18±4	4±1	73±12
	48 h	2.39	342±29	81±8	49±6	27±5	8±4	175±23
20	24 h	3.69	143±15	38±9	14±4	13±4	14±5	66±10
(+)	72 h	5.86	66,5	8.5	7.5	13.5	6.5	31.5
7+14 (+)	48 h	4.54	189.5	24	27	17.5	18.5	102.5
Controls		10	<1	0	0	0	0	<1

E = erythroid ; G = granulocytic ; M = megacaryocytic ; Mx = mixed ; B = blast
PH = phenylhydrazine.
Each value, mean±SE, of four mice
(+) : simple arithmetic mean

After the first days of treatment, the onset of hematopoietic colonies formation was observed : some mononucleated cells with a basophilic

cytoplasm penetrared into Disse's space around Kupffer's cells overloaded with siderosomes. They entered into division, became numerous and extended in the hepatic territories. The hematopoietic colonies in the liver were of various types. The blast type of colony was made up of basophilic cells with large euchromatic nuclei. This colony type had not yet begun its differentiation process.

The differentiated colonies were formed of cells belonging to a blood lineage. Frequently, pure erythroid colonies, consisting of erythroblasts, may be seen. Also pure granulocytic colonies were present . The colonies were often made up of a mixture of erythroid cells and myelocytes. There were mixed colonies. Between the cords of hepatocytes large cells with polyploid nuclei, the megakaryocytes may be observed.

The hematopoietic colonies developed in the portal space area, in the hepatic lobules and in the subcapsular areas.

In the experimental groups in which 3, 7, and 14 inoculations were made (Table 1) the main colony types showed a linear development (either in an increasing or in a decreasing direction). There were recorded significant differences between the number of colonies of any type counted in the liver 48 h after the cessation of the tratment and the colony number found when the animals were sacrificed after 24 h from the last inoculation.

During the first inoculations a continuous coordination between the number of erythroid colonies and the that of blast colonies was noted. As the tratment was continued a lowering of the number of both categories of colonies was recorded. At the same time the number of granulocytic colonies was slightly increased. The megakaryocytic colonies showed a somewhat different development, probably related by own differentiation stimuli. Likewise, the category of mixed colony did not range within a linear development.

A significant lowering of the number of colonies of any type was noted in the animals which survived 20 inoculations. The same observation was recorded in the group of animals in which the treatment was continued after a pause.

The electron microscopic observations confirm the existence of the hematopoietic colony types. All these colonies were sitted in the hepatic cords outside the sinusoids. Frequently pure erythroid colonies were found. Some of them were made up of a smaller number of cells, whereas others were widespread, as a result both of the multiplication of the component cells or of the confluence of several colonies (Fig. 1). In an erythroid colony, the constituent cells were organized around a Kupffer cell. Usually, Kupffer cells contained numerous siderosomes in their cytoplasm. Between the cells of the colony and the Kupffer cell there were close membrane relationships. The cells making up these colonies were erythroblasts, most frequentlly synchronous. The erythroblasts of the various erythroid or mixed colonies (Fig. 2) may be either very young or more advanced in differentiation. Their typical morphological feature was the aspect of the cytoplasm, namely a certain density due to the degree of loading with hemoglobin of the cell stroma. In addition, the erythroblasts were characterized by a small number of organelles : small sized mitocondria and a few endoplasmic reticulum tubules. The young erythroblasts were marked by a density of ribosomes in the cytoplasm. The nuclei of these erythroblasts had a chro-

matin with a coarse drawing and nucleoli. The erythroblasts in a more advanced stage had still fewer organelles and a condensed, diminished, even pycnotic nucleus.

The cells of the pure granulocytic colonies were synchronous (Fig. 3). They had close membrane relationships with the macrophages around which they differentiated. Some colonies were made up of very young cell precursors, of which granulocytes will differentiate. These cells displayed morphological features of myeloid precursors : a large slightly curved nucleus, a rough endoplasmic reticulum, membraneous vesicles, fine granulations, centrioles.

The cells of which megakaryocytes will differentiate were characterized by the presence of a massive or lobate nucleus, a well-developed smooth endoplasmic reticulum and numerous large-sized secretory granules (Fig. 4).

The hematopoietic colonies were always placed in Disse's space (Figs. 1, 2, 3, 4). The cells presented a multitude of fine cellular processes. As a colony increased, it was deeper pushed into the territory of the liver epithelium. The cells of the colony spared the cell membrane of the adjacent hepatocytes. If the hepatocytes were damaged („dark" hepatocytes) (Fig. 1), a cell dislocation can occur under the presence of the hematopoietic colony.

DISCUSSION

During the phenylhydrazine treatment, a hemolytic anaemia occurred in which the loss of erythrocytes exceeded the compensation capacity of the red marrow (17), (23). The anaemia developed following this treatment had as a consequence the stimulation of the erythropoietin secretion (7), (18). (22). The high erythropoietin levels may be associated with the diminution of the normal hematogenous marrow-blood barrier and with the premature release of young, poor differentiated elements into the circulation (5) , (10), (26).

The appearance of blood islands in the liver of mice treated with phenylhydrazine was a significant phenomenon, since this process had never occurred in controls (Table 1).

In contrast with other authors (13), (14), (20) who performed the treatment in a slow rhythm by weekly phenylhydrazine inoculations in this experiment the treatment caused a whipping of the medullary response. The high frequency of stimuli led to a lowering of the medullary response, especially in the experimental group in which 20 phenylhydrazine inoculations were performed. There was a tendency to exhaustion of the medullary resource of stem cells.

The appearance of differentiated types of colonies in the liver was a proof of the existence of local microenvironment conditions for hematopoietic differentiation (6). The erythroid differentiation was provided by

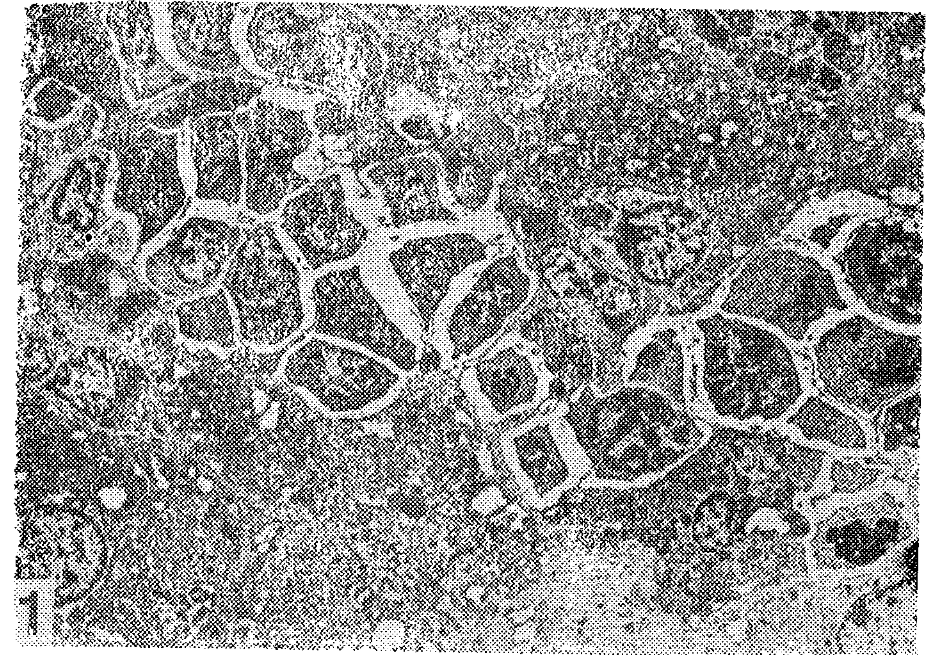

Fig. 1. — Pure colony of erythroid cells in the mouse liver. Erythroblasts are arranged around-Kupffer cells overloaded with siderosomes. 2,400*

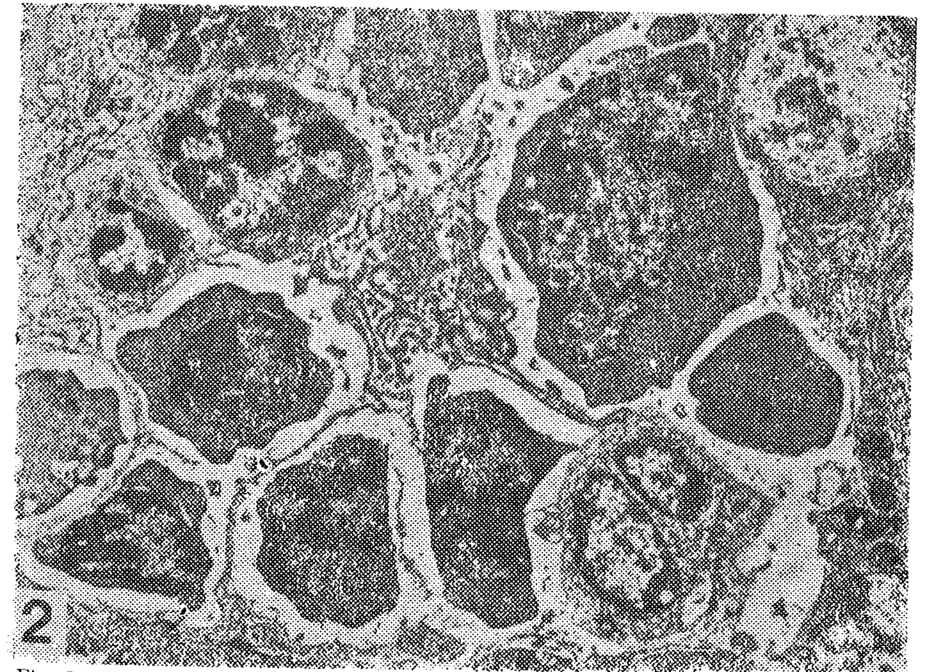

Fig. 2. — Mixed colony. Note the numerous synchronous erythroblasts and a cell in granulocytic differentiation which presents two nuclear profiles and the cytoplasm charged with fine granules and membraneous vesicles. The colony cells are in close relation with the lamellar processes of the Disse's space. 6,000 *

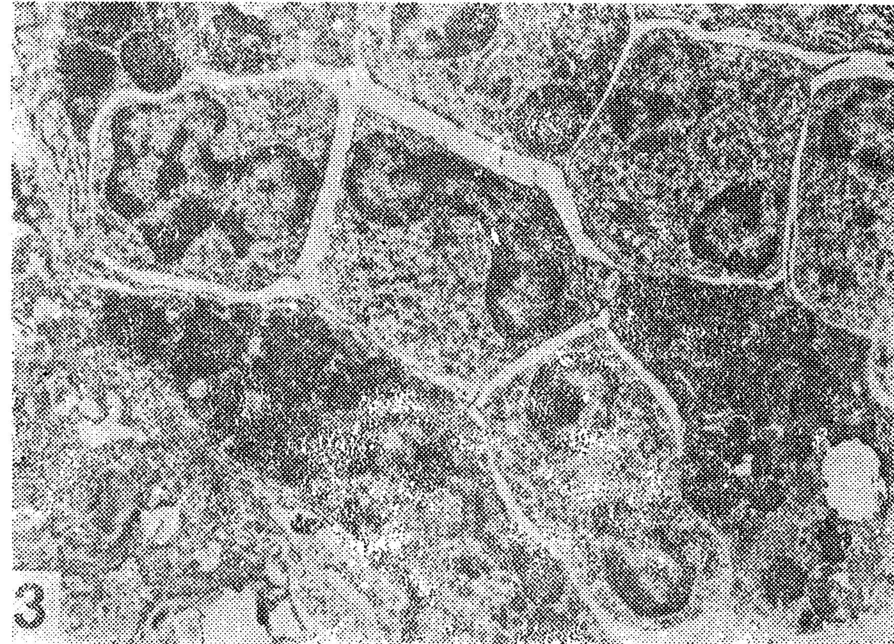

Fig. 3. — Granulocytic colony near Kupffer cells overloaded with the remnants of the destroyed erythrocytes. The synchronism of the myelocytes in the colony is evident. 6,000*

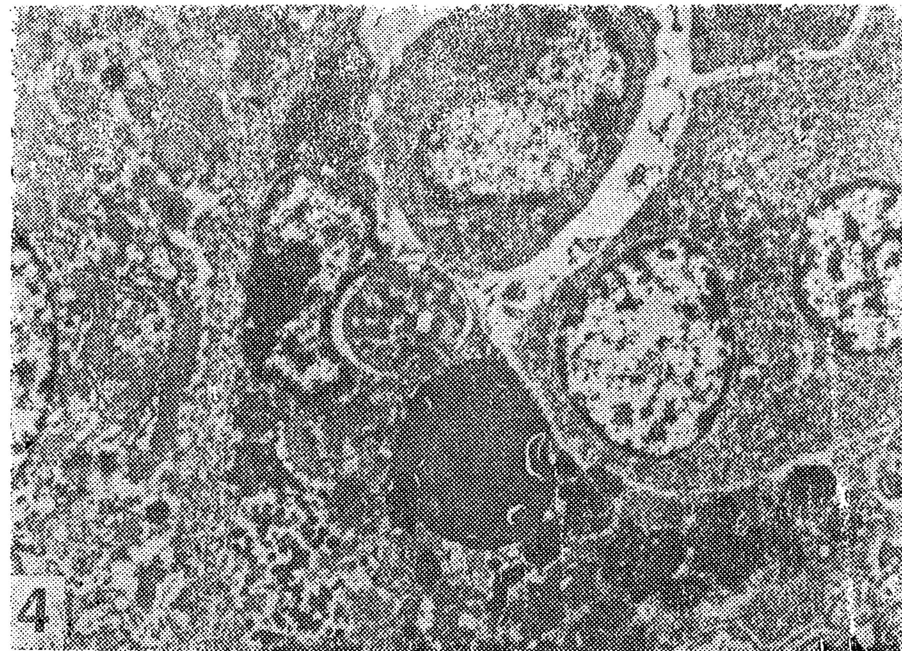

Fig. 4. — Precursor cell of the megakaryocyte, characterized by a large incurved nucleus, an abundant endoplasmic reticulum, large-sized secretory granules. This cell represents close membrane relationshipys with a macrophage overloaded with debris of wasted erythrocytes, siderosomes and ferritin. 7.100*

the relation erythroid colony — Kupffer cells overloaded with ferritin. The Kupffer cell assumed the role of the nurse cell (3). Another factor favouring the hepatic erythropoiesis seemed to be the local tissular hypoxia.

The granulocytic differentiation was probably stimulated by the accumulation in the microenvironment of the products resulted from the phenylhydrazine metabolism. Modern "in vivo" and "in vitro" research, (9), demonstrated the specific activity of some granulocytic stimulation factors.

The hepatic megakaryocyte was considered as originating from a stem cell and as equivalent to a whole colony (13). The megakaryocytic differentiation was promoted by the presence in excess of some intermediary compounds of the hemoglobin metabolism (24). This condition related to the hemolysis produced by phenylhydrazine occurred in the present experiment.

The presence of mixed colonies could plead for the implantation in the liver parenchyma of the circulating pluripotent stem cell (1), (12). However, it could be possible that such colony types resulted from the union of two or several pure colonies deriving from committed stem cells (8).

Our ultastructural observations revealed some data on the features of hematopoietic colonies experimentally obtained in the liver of mice :

— The hepatic hematopietic colonies always developed in close relation with one or several Kupffer cells which intensely exerted their phagocytic functions. Kupffer cells seemed to represent a modulator of the cellular microenvironment.

— The hepatic hematopoietic colonies developed in the territory of the Disse's space. The cells with hematopoietic potentialities penetrated into Disse's space from the blood stream, had an active phase of multiplication, leading to the appearance of extravascular colonies. The cells of the hepatic hematopoietic colonies were often synchronous.

— The cells of the hepatic hematopoetic colonies always maintained close membrane relationships with the surrounding hepatocytes.

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INFLUENCE OF SOME ACEXAMYC COMPOUNDS ON EXTRACELLULAR AND INTRACELLULAR DISTRIBUTION OF WATER AND IONS

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The effects of the acexamyc acid, $ZnSO_4$, $MgSO_4$, ACX-1354 and ACX-1358 derivatives (Zn and Mg, respectivelly, salts of the acexamyc acid) on the total content and distribution of water and Na^+, K^+, Ca^{2+} ions in extracellular and intracellular compartments of the muscle tissue „in vitro" incubated with these agents, were investigated. The results highlighted specific modifications induced by the studied derivatives, both of water and ions total content and of their distribution, as compared to the control agents. These effects allow to consider the acexamyc compounds as membranotropic agents with a permeabilizing action of the cell membrane.

The importance of the cell membranes in maintaining the cellular morphological and physiological integrity, by modulation of exchanges due to their interaction with different agents, the involvement of the membrane modifications in the ethiology of some diseases justify the membranology researches which follow the elaboration of certain therapies by the reestablishment of the membranary normal state (1), (2), (3), (8), (9).

The protective effect of some acexamyc acid dervivatives (6) (12) and Zn salts to ulcerogenic action of certain agents on gastric mucous membrane suggested us to investigate their probably action mechanism.

In the present paper, the interaction of some acexamyc derivatives with cellular membrane is exposed, by studying their specific effects on tissue repartition of water and of Na, K^+ and Ca^{2+} ions in extra- and intracellular compartiments.

MATERIALS AND METHODS

"In vitro" action of ACX-1354 (Zn acexamate) and ACX-1358 (Mg acexamate) compounds was studied as compared to that of the acexamyc acid as well as of ZnSO and MgSO – because of the acexamyc anion and Zn^{2+} and Mg^{2+} cations present in the derivative structure – added to the medium in a dose of 0.6g/100 ml.

The experiments were performed on sartorius muscles isolated from *Rana ridibunda* Pall frog and " in vitro" incubated for three hours at room temperature in normal Ringer physiological solution (pH 7.2) containing or not the studied agents. Each experimental series was realized on minimum five animals.

Total water content (g/100 g fresh tissue) was determined by the difference between the fresh tissue weight and that one after drying at 105°C. Starting from the dried and mineralized tissue, the total amount of Na^+, K^+, Ca^{2+} ions (mg/100 g fress tissue) was assessed by flamphotometrical method (7).

The extracellular space was estimated by the inulinic space method (4) incubating the treated and control muscles in Ringer solution with 1% inuline. The intracellular space was evaluated by the difference between total water volume and that one of the extracellular water.

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The extra- and intracellular ionic contents (mg/100 g fresh tissue) were calculated taking into account the extra- and intracellular water amounts, the extracellular ionic concentration equivalent with that one from incubating Ringer medium as well as the total ionic quantity.

The statistic significance was established using Student's "t" test (4).

RESULTS

"In vitro" incubating of the sartorius muscle in Ringer solution, containing ACX—1354 or ACX—1358 products, acexamyc acid, $ZnSO_4$ or $MgSO_4$, was correlated with modifications both of the total water and Na^+, K^+, Ca^{2+} ions content and of their distribution in the extracellular and intracellular compartiments.

The experimental results are presented in Tables 1—4, they reflecting the water and ionic content variations from tissular compartments under the treatment influence as compared with the untreated control.

DISCUSSIONS

Numerous researches emphasizéd that the cell membrane represents the preferential target of the action of a wide range of active biological agents, their action mechanism being conditioned by the membrane structural and functional particularities as well as by the specific structure of these membranotropic agents (1), (3), (9), (10).

In certain diseases there were registered modifications of some morphophysiological parameters of the cellular membranes, being evidented the positive effect of the agents which restore the normal functional state of membranes (1), (2), (5), (8), (9).

Thus, it was observed the protective effect of some acexamyc acid derivatives in the case of ulcerogenic action of certain agents of the gastric mucous membrane (6), (12).

The cell membrane implications in the expression of the intracellular effect of some membranotropic agents justified the investigation of the ACX-1354 and ACX-1358 acexamyc derivatives action at the membranary level by following their specific effects on water and Na^+, K^+, Ca^{2+} ions repartition in the extra- and intracellular compartments, comparatively with the action of acexamyc acid, Zn^{2+} and Mg^{2+} which are in the chemical structure of these derivatives.

The analysis of the obtained results highlighted that all studied agents induced specific modifications in the extra- and intracelular repartition of water and ions, compared to the untreated control, suggesting an action of membranary permeabilisation.

Thus, it can be seen that the ACX-1354 and ACX-1358 induced a significant increase of the extracellular water volume (50.7% and 67.3%, respectively, as compared to the control), which was correlated with an adequate decrease of the intracellular one (11.6% and 21.7%, respectively). The total water amount increased easily (1.8%) under the action of ACX-1354 product and decreased (2.9%) to ACX-1354 derivative action

(Table 1). The acexamyc acid and $MgSO_4$ effects are generally similar to that one of the ACX-1358 compound, while the action of $ZnSO_4$ is different, it determining easy augmentations of the water content in all compartments.

Therefore, it can be appreciated that the specific effects on water distribution are due the convergent action both of the acid component and of the ion form the structure of derivatives.

All studied agents induce identical modifications of the membrane permeability for Na^+ (Table 2). Thus, it is observed an increase of Na^+ extracellular content (11.5% at ACX-1354 and 8.9% at ACX-1358), correlated with decreases of the intracellular one (52.4% at ACX -1354 and 86.2% at ACX-1358) and of the total amount (19.8% at ACX-1354 and

Table 1

Water total content (g/100 g wet weight) and water distribution in the muscle tissue, incubated with different agents (0.6 g%). Figures in brackets indicate the experiment number

Treatment	Repartition ($\bar{x}\pm$ES)		
	Total	Extracellular	Intracellular
Control	80.91±0.32(5)	17.11±0.68(5)	63.83±0.61(5)
ACX-1354	82.40±0.33(5) p / 0.02	25.78±0.48(5) p < 0.001	56.42±0.90(5) p < 0.001
$ZnSO_4$	82.77±0.20(5) p / 0.002	18.56±(1.055) N.S.	64.21±1.00(5) N.S.
ACX-1358	78.64±0.24(5) p / 0.001	28.63±0.35(5) p < 0.001	50.01±0.55(5) p < 0.001
$MgSO_4$	79.31±0.21(5) p / 0.01	27.32±0.26(5) p < 0.001	51.99±0.33(5) p < 0.001
Acexamyc acid	79.10±0.38(5) p / 0.01	32.20±1.24(5) p < 0.001	46.90±1.48(5) p < 0.001

Table 2

Total, extracellular and intracellular Na^+ (mg/100 g wet veight) in the muscle tissue incubated with different agents 0.6 g%).

Treatment	Repartition ($\bar{x}\pm$ ES)		
	Total	Extracelular	Intracellular
Control	89.72±4.79(5)	45.84±1.84(5)	43.88±4.10(5)
ACX-1354	71.95±3.33(5) p < 0.02	51.07±0.95(5) p < 0.01	20.88±2.52(5) p < 0.002
$ZnSO_4$	86.94±4.16(5) N.S.	46.85±2.63(5) N.S.	40.19±3.90(5) N.S.
ACX-1358	55.98±0.57(5) p < 0.001	49.93±0.60(5) p < 0.01	6.05±0.79(5) p < 0.001
$MgSO_4$	79.31±3.09(5) N.S.	55.05±0.52(4) p / 0.001	24.26±2.13(5) p < 0.01
Acexamyc acid	89.45±4.83(5) N.S.	63.70±2.45(5) p < 0.001	25.75±3.99(5) p < 0.02

37.6 % at ACX-1358). Similar variations were determined by acexamyc acid, $ZnSO_4$ and $MgSO_4$ actions, but the $ZnSO_4$ effects were weak. Thus, it is ascertained that all agents induce a depletion of Na^+ from the cell.

It can be concluded that in the case of ACX-1354 product, the responsible factor for the effects on Na^+ would be the acid radical from the structure of the molecule. The acexamyc acid effects are stronger than those of the $ZnSO_4$. In the case of ACX-1358 derivative the effects on Na^+ would be due to the both molecular components, but Mg^{2+} seems to be prevalently in determining the Na^+ depletion.

The ACX-1354 compound modifies the membrane permeability for K^+ (Table 3), which leads to a decrease of K^+ intracellular content (47.8 %,

Table 3

Total, extracellular and intracellular K^+ (mg/100 g wet weight) in muscle tissue incubated with different agents (0.6 g %)

Treatment	Repartition ($\bar{x}\pm ES$)		
	Total	Extracellular	Intracelullar
Control	329.67±8.07(5)	1.67±0.07(5)	327.90±8.09(5)
ACX-1354	173.82±8.89(5) $p < 0.001$	2.52±0.05(5) $p < 0.001$	171.30±9.92(5) $p < 0.001$
$ZnSO_4$	134.81±7.81(5) $p < 0.001$	1.81±0.10(5) N.S.	133.00±7.90(5) $p < 0.01$
ACX-1358	367.87±21.82(5) N.S.	2.80±0.03(5) $p < 0.001$	365.07±21.54(5) N.S.
$MgSO_4$	348.34±12.71(5) N.S.	2.67±0.04(5) $p < 0.001$	345.67±12.47(5) N.S.
Axcexamyc acid	171.62±9.46(5) $p < 0.001$	3.14±0.13(5) $p < 0.001$	168.48±9.38(5) $p \leqslant 0.001$

as compared to the control), concomitantly with an increase of the extracellular amount (50.9 %) and a decrease of the total one (47.3 %), thus registering an important depletion of K^+. Same modifications of the K^+ ion were induced by the acexamyc acid and $ZnSO_4$. Probably the effect of the ACX-1354 derivative on K^+ represents the result of the convergent actions of both components from its molecule.

The ACX-1358 compound action determines different effects, leading to the augmentation of K^+ content in all compartiments: intracellular (11.3 %), extracellular (67.7 %) and total (11.6 %) on the basis of a K^+ influx from the incubating medium. Similar modifications were also registered in the $MgSO_4$ action. These data suggest that the action of the product on K^+ is due to Mg^{2+} ion from its molecule and not to acexamyc radical, which has different effects.

The effects of both derivatives on Ca^{2+} distribution were also different (Table 4). Thus, ACX-1354 indreed an increase of the Ca^{2+} content in all compartiments: intracellular (38.5 %), extracellular (50.3 %) and total (40.6 %), this being justified by an influx from the incubating medium. The acexamyc acid and $ZnSO_4$ were characterized by the same effects,

but in the case of the last they were much weak. These results allow to consider that the action of ACX-derivative on Ca^{2+} dynamics would be mainly due to the acid component from its molecule. The ACX product presented different effects on Ca^{2+} repartition: a decrease of the intracellular amounts (15.7 %), an increase of the extracellular one (66.7 %) and

Table 4

Total, extracellular and intracellular Ca^{2+} (mg/100 g wet weight) in the muscle tissue incubated with different agents (0.6 g %)

Treatment	Reparatition ($\bar{x}\pm ES$)		
	Total	Extracelullar	Intracelullar
Control	7.90±0.47(5)	1.41±0.06(5)	6.49±0.52(5)
ACX-1354	11.11±0.97(5) $p < 0.02$	2.12±0.04(5) $p < 0.001$	8.99±0.99(5) $p = 0.05$
$ZnSO_4$	8.95±0.88(5) N.S.	1.52±0.08(5) N.S.	7.43±0.93(5) N.S.
ACX-1358	7.82±0.43(5) N.S.	2.35±0.03(5) $p < 0.001$	5.47±0.45(5) N.S.
$MgSO_4$	8.50±0.37(5) N.S.	2.24±0.02(5) $p < 0.001$	6.26±0.38(5) N.S.
Acexamyc acid	10.37±0.71(5) $p < 0.02$	2.64±0.11(5) $p < 0.001$	7.73±0.79(5) N.S.

a very slight decrease of the total content (1.0 %). Similar effects were registered to $MgSO_4$ action. It can be concluded that the ACX-1358 derivative action on Ca^{2+} represents the consequence of the presence of Mg^{2+} in its structure.

The bulk of the experimental data revealed that the acexamyc derivatives exert a strong influence on the membrane permeability for water and ions, modifying their distribution in the tissue compartments. Thus, they can be characterized as membranotropic agents, which induce a new level of the extra- and intracellular ionic concentration as well as of the ratio between the ions.

These modifications determined by the acexamyc derivatives allow to presume positive influences on the cell metabolic processes, the enzymatic activity and the aminoacids and glucose transport (1), (9), (10), with implications on their antiulcerous therapeutic effect (6), (12).

The membranary action of the acexamyc derivatives imposes the investigation of their effects on the water and ions transmembranary fluxes, the ionic pumps activity, the report between the active transport and the passive one, as well as on the intra- and extracellular ionic ratios. Thus, there would be evidentiated the specific interaction of these agents with the cell membrane and the membranary mechanisms involved in the inducing of their specific action.

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THE ACTION OF SOME ACEXAMYC DERIVATIVES ON WATER AND IONS TRANSMEMBRANARY DYNAMICS

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The influence of acexamyc derivatives ACX—1354 and ACX-1358 was investigated, as compared to that one of their structural components (acid, Zn and Mg), on hydrous and ionic transmembranary fluxes, extra- and intracellular ionic ratios, as well as on the involvement of the active and passive transport mechanisms. The modifications of the water and Na^+, K^+ and Ca^{2+} ions dynamics, argued by the variations of their quantitative distribution in the tissue compartments, of the extra- and intracellular ionic ratios — determined by the amplitude of the hydrous and ionic influxes and effluxes, as well as of the proportion between the active and passive mechanisms of the transmembranary transport — confirm the membranotropic action of the Zn and Mg acexamates. These seem to behave as hyperpolarizing and stabilizing membranary agents, explaining, thus, their recorded effect of membrane protection.

The effects of Zn and Mg acexametes on cell membrane permeability, illustrated by the modifications of water and Na^+, K^+, Ca^{2+} ions distribution in extra- and intracellular compartments, were previously evidenced (9).

The characterization of these compounds as membranotropic agents imposes the following of their interaction with the cell membrane and of the membranary mechanism involved in inducing their specific action (4), (16), (17).

In the present paper there are induced the results obtained in the investigation of the ACX-1354 and ACX-1358 acexamyc derivatives influence on water and ions transmembranary fluxes as well as of their consequences on extra- and intracellular ionic ratios.

MATERIAL AND METHODS

The experiments were performed on sartorius muscles isolated from *Rana ridibunda* Pall. frog and "in vitro" incubated three hours at room temperature in Ringer physiological solution (pH 7.2) containing or not 0.6 g/100ml of acexamyc products; ACX-1354 (Zn acexamate) and ACX-1358 (Mg acexamate). For an objective appreciation of their effects, the action of acexamyc acid as well as of $ZnSO_4$ and $Mg\ SO_4$ were also followed in the same experimental conditions, in relation to the presence of acexamyc anion and Zn and Mg cations in the studied derivatives structure.

The estimation of water and ions transmembranary fluxes was based on the registering of their distribution in extra-and intracellular compartments. The total water volume (ml/100 g fresh tissue) was calculated by the difference between the tissue weight before and after drying at 105 °C. The extracellular water amount was assessed by inulinic space method (8) both for treated and untreated muscles. The intracellular space was established by the difference between the total water volume and the one extracellular.

The total concentration (mg/100 ml total tissue water) of Na^+, K^+ and Ca^{2+} ions was calculated on the basis of total tissue ionic content (mg/100 g fresh tissue) determined by the flamphotometrical method (13). Ionic content (mg/100 ml tissue water) form intracellular compartment was appreciated taking into account the intracellular water volume, the extracellular ionic concentration equivalent with that from incubating Ringer solutions, as well as the total ionic amount.

The experimental results, included in the tables, were estimated on the basis of the average values of water and ions contents (mg/100 g fresh tissue) presented in a previous work (9).

RESULTS

"In vitro" treatment of the sartorius muscle in Ringer solution containing Zn and Mg acexamates, as well as acexamyc acid, $ZnSO_4$ or $MgSO_4$ (0.6g/100 ml), was correlated with some modifications of the water transmembranary dynamics. These are suggested by the values of total, extra- and intracellular hydrous volumes (Table 1).

Table 1

Total, extra- and intracellular water contents (%) of the fresh muscle tissue incubated with different agents (0.6g/ 100 ml). Figures in brackets indicate the experiments number

Treatment	Distribution (%)		
	Total	Extracellular	Intracellular
CONTROL	100.00(5)	100.00(5)	100.00(5)
ACX-1354	101.80(5)	150.70(5)	88.40(5)
$ZnSO_4$	102.30(5)	108.50(5)	100.60(5)
ACX-1358	97.10(5)	167.30(5)	78.30(5)
$MgSO_4$	98.00(5)	159.70(5)	81.40(5)
Acexamyc acid	97.30(5)	188.10(5)	73.40(5)

The variations of the total, extra- and intracellular ionic concentrations (mg/100 ml water), registered in the same experimental conditions, are illustrated by the data included in Tables 2–4.

Table 2

Na^+ ion dinamyes in the fresh tissue incubated with different agents (0.6g/100 ml)

Treatment	Ion distribution (mg/100 ml water)		
	Total	Extracellular	Intracellular
CONTROL	110.90	267.92	68.79
ACX-1354	87.32	267.92	32.96
$ZnSO_4$	105.04	267.92	61.53
ACX -1358	71.18	267.92	10.08
$MgSO_4$	100.00	267.92	41.28
Acexamyc acid	113.08	267.92	46.54

Table 3

K^+ ion dynamics in the fresh tissue incubated with different agents (0.6g/100 ml)

Treatment	Ion Distribution (mg/100 ml water)		
	Total	Extracellular	Intracellular
CONTROL	407.38	9.77	513.71
ACX-1354	210.95	9.77	271.49
$ZnSO_4$	162.87	9.77	203.78
ACX-1358	467.70	9.77	610.08
$MgSO_4$	439.21	9.77	584.30
Acexamyc acid	216.96	9.77	305.98

Table 4

Ca^{2+} ion dynamics in the fresh tissue incubated with different agents (0.6g/100 ml)

Treatment	Ion distribution (mg/100 ml water)		
	Total	Extracellular	Intracellular
Control	9.76	8.22	10.16
ACX-1354	13.48	8.22	15.07
$ZnSO_4$	10.81	8.22	11.51
ACX-1358	9.94	8.22	10.48
$MgSO_4$	10.72	8.22	11.59
Acexamyc acid	13.11	8.22	15.22

Table 4

Tissue ionic ratios characteristic for the fresh muscle tissue incubated with different agents

Treatment	Ionic Ratios					
	Water E / Water I	Na^+E / Na^+I	K^+E / K^+I	$Ca^{2+}E$ / $Ca^{2+}I$	Na^+E / K^+I	K^+I / Na^+I
Control	1.00	3.89	0.019	0.81	0.52	7.47
ACX-1354	1.70	8.13	0.036	0.54	0.99	8.24
$ZnsSO_4$	1.08	4.35	0.048	0.71	1.31	3.31
ACX-1358	2.14	26.58	0.016	0.78	0.44	60.52
$MgSO_4$	1.96	6.49	0.017	0.71	0.45	14.28
Acexamyc	2.56	5.76	0.032	0.54	0.87	6.57

Also, the ionic concentrations modifications allow to calculate the ionic rations, either for the same ionic species or between different ions, these being presented in Table 5.

"In vitro" incubating of the sartorius muscles with the ACX-1354 derivative determined modifications in water and ions distribution in the tissue compartments comparatively with that one registered in untreated control. Thus, the total and extracellular water volumes increase with 1.8% and 50.7% respectively, on the basis of intracellular volume decrease (11.6%). The Na^+ total concentration presents a significant decrease with 21.3%, explained by a corresponding diminuation (52.1%) of the intracellular Na^+. A similar effect is observed at K^+, which touches decreased levels with 44.2%, in the case of the total one and with 47,1%, in the case of the intracellular one, the K^+ efflux in medium justifying this behaviour of the ion. On the contrary, the Ca^{2+} concentration, both total and intracellular, records an increase of 38.1% and 48.3%, respectively.

The addition of $ZnSO_4$ in the incubating medium leads to: slight increases of the total (2.3%), extracellular (8.5%) and intracellular (0.6%) water content; decreases with 5.3% and 10.5% of the total an dintracellular Na^+ concentration; strong diminutions of the total (60%) and intracellular Ca^{2+} of 10.8% and 13.3%, respectively.

Modifications of the hydrous and ionic dynamics were also induced by the treatment with ACX-1358 derivative. Thus, concomitantly with a slight decrease of the total water volume (2.9%), it was found a great increase of the extracellular water (67.3%), correlated with a diminution of the intracellular one (21.7%). Both total and intracellular Na^+ presents ample reductions of 35.8% and 85.3%, respectively. Contrarily, the total and intracellular values of K^+ reflect augmentations of 14.8% and 18.8% respectively. A similar phenomenon, but of small proportion, characterizes the Ca^{2+} ion (total increases with 1.8%; intracellular ones with 3.1%).

$MgSO_4$ induces variations of water and ions repartition in the tissue compartments, as follows: the water presents a decrease of the total volume (2%) and of the intracelullar one (18.6%), correlated with an increase of the extracellular hydrous content (59.7%); Na^+ decreases: total with 9.8%, intracelullar with 40.9%; K^+ increases: total with 7.8%, intracellular with 14.7%; Ca^{2+} enhances: total with 9.8%, intracellular with 14.1%.

Finally, the treatment of the sartorius muscle with acexamyc acid— the main constituent of the studied derivatives — determines a specific distribution of water and ions. Thus, the total and intracellular water registers decreases of 2.7% and 26.6% respectively, while the extracellular volume strongly increases with 88.1%. In the context of maintaining a total Na^+ relative constant concentration (only + 1.9%), it is found a significant diminution of intracellular Na^+ (32. 3%). A different situation is observed in the case of K^+, its total and intracellular concentrations greatly decreasing with 46.7% and 40.4%, respectively. The Ca^{2+} ion dynamics is marked by ample increases of the total concentrations (34.3%) and of the intracellular one (49.8%).

At the same time, it is established, as compared to the control, a reduction of the total intracellular ionic concentration of $Na^+ + K^+ + Ca^{2+}$ in the case of ACX-1354 (46.1%), $ZnSO_4$ (53.3%) and of acexamyc acid (38.0%) agents, as well as an augmentation of this, in the case of ACX-1358 (6.4%) and $MgSO_4$ (8.4%) agents. Reporting the total ionic intracellular concentration to the water intracellular volume it is ascertained a decrease of the osmotic concentration of 38.7% with ACX-1354 of 53.8% with $ZnSO_4$ and of 30.1% with acexamyc acid, as well as an increase of this in the case of ACX-1358 (35.5%) and $MgSO_4$ (32.3%).

DISCUSSION

The steady state of the membrane or its proper resting state expresses the normal molecular and supramolecular organization of this, highlighted in the opening or packing degree of the membranary structures, as well as in the water and ions fluxes corresponding to this state (1), (4), (16), (17).

The membranotropic, agents, by interaction with the cell membrane, induce structural modifications of the membranary components (stabilization, labilization, fluidization, modifications of the molecular conformation and of micellar organization) with consequences on membrane physiology, disturbing the water and ions transmembranary fluxes (3), (16).

The evidentiation of the membranary permeabilizing action (9) suggested us to appreciate the effect of ACX-1354 and ACX-1358 acexamyc derivatives on the hydrous and ionic transmembranary fluxes, ionic pumps activity, proportion between active and passive transport, as well as on the intra- and extracellular ionic ratios. Thus, it would be highlighted the interaction of these agents with the cell membrane and the membranary mechanisms involved in inducing their specific action.

The analysis of the Na^+ ion dynamics in the muscle tissue treated with the studied agents reveals — as a general characteristic — the modification of the transmembranary flux, represented by the increase of Na^+ efflux. The amplitude of the intracellular Na^+ depletion differs, touching the maximum level in the case of ACX-1358 derivative.

Because of the constant extracellular Na^+ concentration in the incubating Ringer medium, the modification of the Na^+ transmembranary transfer leads to a change of the Na^+E/Na^+I ionic ratio. These suggest the involvement of an active ionic transport mechanism, Na^+ being excluded from the cell as against the concentration gradient. The stimulation of Na^+ pump can be correlated with a probable reduction of Na^+ passive transport, too (15), (16).

It can be also observed that the Na^+ increased efflux imposes the water passive outflow. The water osmotic flux determines the diminution of the cell volume, which is however compatible with the normal unfolding of the cellular processes. The modification of the water distribution can be the result of the agents action both on the osmotic way — because in all cases it was registered an increase of the extracellular water content — and on the way of "water pump" (11), (14). The increase of the water flux through membranes is also accompanied by the enlargement of the flux of some ions on the basis of the "solvent drag" mechanism [7], [11], this being previously signaled in the case of Na^+.

The effects on K^+ distribution are not similar with those on Na^+ repartition in the case of ACX-1358 product, registering an increase of the K^+ influx. This reflects an enhancement of the K^+ active transport and/or the diminution of its passive transport. The increased intracellular con-

centration of the K^+ can be also explained by the water intracellular reduction.

The specific dynamics of K^+ is also argued by the decrease of the K^+E/K^+I ionic ratio. The significance of the ionic ratios Na^+ E/K^+I and K^+I/Na^+I consists in the increase of the cell membrane electrical charge under the action of this agent. The membrane hyperpolarization, which touches an amplitude of 10 mV, after 15 minutes (unpublished data) suggests an activation of the electrogenic Na^+—K^+ pump.

In the case of the ACX-1354 product it is observed an inverse situation. The large depletion of the intracellular K^+, correlated with the increase of the K^+E/K^+I ratio, suggests the decrease of the transmembranary active transport mechanism effectiveness and/or the increase of the membrane passive permeability for K^+. Although the K^+ efflux increases, this is smaller than Na^+ depletion, a proof being the enlargement of the K^+I/Na^+I ratio. These illustrate an effect of membrane hyperpolarization (5.8 mV after 15 minutes), mainly determined by the Na^+ active efflux.

The Ca^{2+} distribution is also influenced by the investigated agents, the total content and the influx increasing in all cases, the amplitude of the modifications being differently. This transmembranary dynamics is also illustrated by the decrease of the $Ca^{2+}E/Ca^{2+}I$ ratio. The influx and ratio values become significant , especially to the ACX-1354 derivative. The increase of the Ca^{2+} intracellular concentration could be also explained by the decrease of the water intracellular volume. Now it is difficult to appreciate the involvement degree of the active and passive mechanisms in the transmembranary fluxes of this ion.

The Ca^{2+} increased intracellular concentration could reduce the intensity of the Na^+ and K^+ active transport by inhibiting the Na^+ -K^+ pump on the membrane internal face [5], [6].

It is known that an increase of the Na^+ intracellular concentration determines an inhibition of Ca^{2+} sequestering at cell structural level [2], [10]. But we ascertained a decrease of the intracellular Na^+, which could lead to the increase of the Ca^{2+} sequestering degree conditioning a diminished level of intracellular ionic Ca^{2+} and by this the noninhibiting of Na^+—K^+ pump activity.

The decrease of the total intracellular ionic concentration of Na^++K^+ + Ca^{2+}, correlated with the reduction of the water intracellular volume, in the case of ACX—1354 product and the increase of the value, in the case of ACX-1358 agent, reveal the directions of the water and ions transmembranary fluxes. Thus, it can be appreciated a predomination of the ionic efflux comparatively with the hydrous one, in the case of ACX-1354 derivative and a predomination of the ionic influx, correlated with a water efflux, in the case of ACX-1358 compound.

The membranotropic action of the Zn and Mg acexamates seems to be the consequence of the interference of the specific effects of components, acid and mineral ones, from the structure of derivatives.

The acexamyc derivatives with membranotropic action seem to behave as hyperpolarizing and stabilizing agents, explaining their membranary protection effect [2], [18]. Also, the acexamyc derivatives could

influence the cell enzymatic and metabolic activity by the establishment of a new level of the extra- and intracellular ionic ratios, which suggests the enhancement of the active transport.

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INVESTIGATION OF THE EFFECT OF SOME PHENOLIC FRACTION ON HeLa CELL CULTURES DEVELOPMENT

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The *in vitro* effect of some phenolic fractions, extracted from Asclepias syriaca leafs, on HeLa cell cultures evolution was tested. The significant modifications of the protein dynamics, considered as an expression of the inhibitory effect, of the fractions on HeLa cell cultures development, allowed the general appreciation that some phenolic structures have cytostatic properties. For the finding of an or some *in vitro* cytostatic agent or cytostatics agents and *in vivo* cancerostatic or cancerostatics a selective separation and a chemical identification of the phenolic substances from the extracted fractions composition are necessary.

The *in vitro* cytostatic and *in vivo* antitumoral actions of some total polyphenolic preparations were previously reported (8), (9).

The structural complexity of these products suggested us to investigate which of the phenolic type structures from *Asclepias syriaca* vegetal extract composition should be responsible for their cytostatic property, as a premise for finally obtaining a product with increased cancerostatic effectiveness by its separation of that inactive from this point of view. This hypothesis is based on our finding that only the chemical removal of the hemicellulosic structures, from the initial vegetal extract composition, conditioned the significant utterance of the PA total polyphenolic preparation cytostatic action (9) . This effect is probably due either to a consecutive increase of the active biological phenolic substances or to a consecutive relieving of the functional groups which were coupled with the hemicellulosic structures (4), (10).

In the present paper the results of the *in vitro* testing of the effect of some phenolic fractions — separated and purified from *Asclepias syriaca* leaves — on HeLa cell cultures development — are exposed.

MATERIAL AND METHODS

The *in vitro* investigated phenolic fractions were noted as Fr. 1, Fr. 2, Fr. 3, Fr. 4, Fr. 6 and Fr. 8. These fractions were separated and purified from October harvested leaves of *Asclepias syriaca* by successive extractions with different solvents (cyclohexane, ethanol, ethyl ether, acetone) and by the removal of the hemicellulosic structures. The various sediments were dissolved in : cyclohexane (Fr. 1a), ethyl acetate (Fr. 1 b), sodium hydroxide (Fr. 2, 4, 6, 8), glycerol diluted with bidistilled water 1 :1 (Fr. 3 a) and bidistilled water (Fr. 3 b).

The cytostatic action was assessed *in vitro* by comparative follow-up of the total protein dynamics during the evolution of the control and treated HeLa cell cultures, as an expression of the cell protein biosynthesis level.

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The test tubes were inoculated with 1×10^5 cells and after 24 hours the culture medium was replaced with a medium containing either 1.5mg/ml of phenolic fractions or, in some cases, corresponding volumes from their solvents. At 24, 48 and 72 hours of cultures development, the medium was discarded from the test tubes and the cell layer was washed with TFS and subjected to total protein determination (6), (7).

Five culture tubes were used for each type of culture and time interval and the statistical analysis was performed using Student's "t" test (15).

The evaluation of cytostatic action was made by the percentage estimation of the inhibitory effect of the cell cultures development illustrated by the decrease of the total protein concentration.

The appreciation of the cytostatic effect was performed by a comparative analysis of our values with that one of reference (50 %) imposed by the selection criteria of antitumoral substances established by the chemotherapeutic screening programs of the Cancer Chemotherapy National Institute from the U.S.A. (5),(13), (14), for this preliminary step.

RESULTS

In a first experiment, illustrated in Fig. 1, the action of the Fr. 1 a and Fr. 1 b phenolic fractions and of their solvents on HeLa cell cultures development was tested.

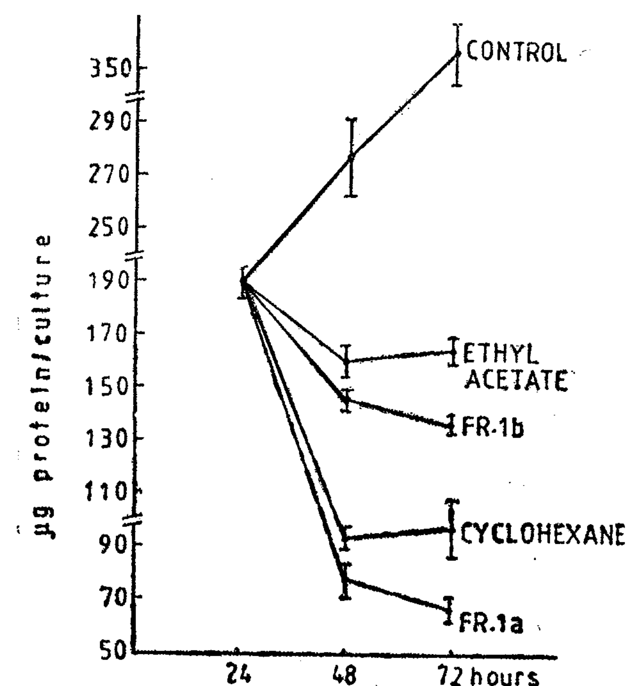

Fig. 1. — Protein content of HeLa cell cultures incubated with Fr.h 1a and Fr. 1 b (1. 5mg/m l).

The cultures incubated with Fr. 1 a were characterized by a rapid, strong and significant ($p<0.001$) decrease of protein concentration, reaching 72.2% and 81.3% respectively, from the normal values of the controls at both intervals analysed. A similar effect was registered at the cultures incubated with cyclohexane, the solvent of the Fr. 1 a. Thus, the protein values determined at 48 and 72 hours registered significant decreases ($p<0.001$) of 66.5% and 72.5% as compared to the controls.

The protein dynamics in the cultures treated with Fr. 1 b showed lower significant levels ($p < 0.001$) of the protein values, as compared to the controls, of 47.3% and 61.3% respectively, at the time interval analysed. A significant cytotoxic action ($p<0.001$) on HeLa cells is also observed with the ethyl acetate — the characteristic solvent of Fr. 1 b — it being illustrated by a decrease of the protein concentrations of 41.7% and and 53.9%, as compared to the control values.

Another set of phenolic fractions tested contained Fr. 2, Fr. 4, Fr. 6 and Fr. 8, the experimental results being included in Fig. 2. It is observed, in all the cases, that the protein dynamics of the treated cultures significantly differ from that one of the controls, reflecting various intensities of inhibition of the proteinosynthesis. Thus, during the whole experimental interval, the decreased protein values revealed inhibitions of the cultures development of :

— 34.8% and 56.6% respectively, in the case of Fr. 2 ;
— 42.2% and 61.2%, respectively, in the case of Fr. 4 ;
— 43.6% and 68.1%, respectively, in the case of Fr. 6 and
— 55.9% and 73.8%, respectively, in the case of Fr. 8.

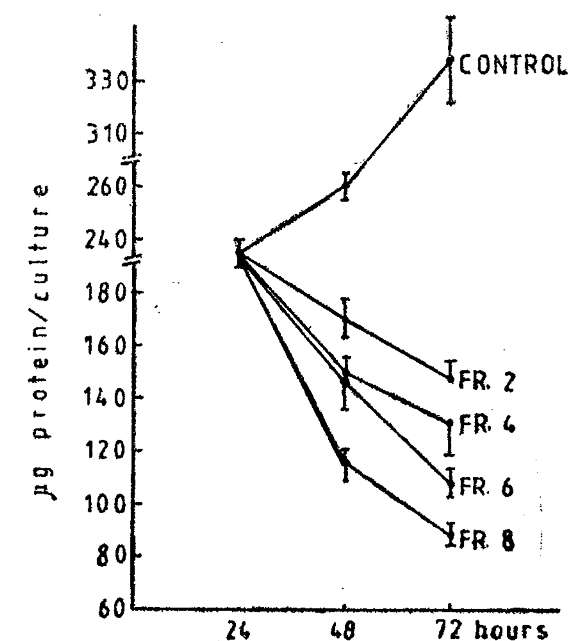

Fig. 2. — Protein dynamics of HeLa cell cultures treated with the fractions 2, 4, 6 and 8 (1.5 mg/ml).

Finally, the prescreening programme included the phenolic fractions 3a, 3b and the water diluted glycerol — the solvent of Fr. 3 a — the experimental data being presented in Fig. 3.

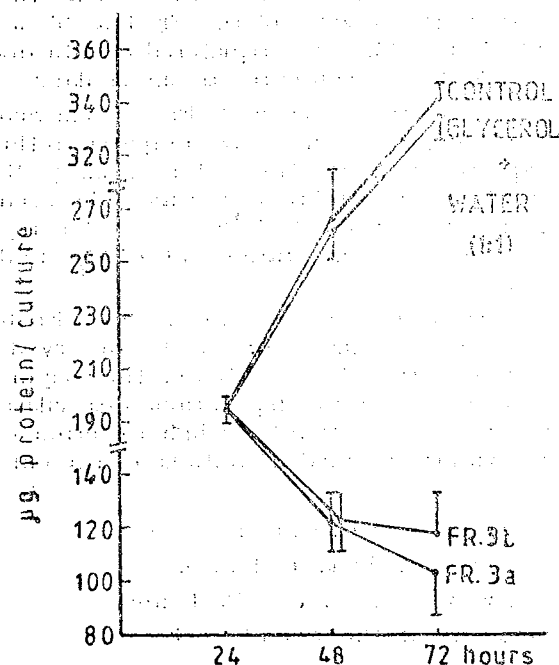

Fig. 3. Protein content of HeLa cell cultures incubated with Fr. 3 a and Fr. 3 b (1.5mg/ml).

It can be seen that, in the both cases, the treated cultures were characterized by a similar protein dynamics, but at the same time differed from that one of the controls. Thus, Fr. 3 a induced decreases of the protein values of 54.3% and 66.3%, respectively. This inhibitory action is unsignificantly influenced by the diluted glycerol, its cytotoxic effect being negligible (1.6 and 1.9 %).

The inhibiting potential of the fraction 3 b was identical with that one of Fr. 3 a, the protein concentrations reaching 53.9% and 65.1% from the normal values of controls.

DISCUSSION

In previous studies we evidenced the *in vitro* cytostatic and *in vivo* cancerostatic actions of the PA_2 product, as well as the higher *in vitro* cytostatic potential of the PA_2 III total polyphenolic preparation (8), (9). These products are total polyphenolic preparations separated and purified from *Asclepias syriaca* leaves. They are characterized by a complex chemical composition containing, probably, beside wax, latex, fatty acids, fatty alcohols, simple saccharides, pectins, and numerous structures of phenolic type such as : lignins, lignanes, terpenoids, phenolic acids, phenolic aldehydes, phenolic esters, acetophenones, hydroxycinnamic acids, phenylpropenes, coumarins, isocoumarins, benzoquinones, naphthoquinones, xanthones, stilbenes, flavonoids, proanthocyanidines /2/, /11/, /12/.

This structural complexity of the vegetal extract imposed its fractionating for the separation of the structure/structures responsible of their cytostatic and cancerostatic actions. Thus, by successive extractions and dissolving of the sediments in different solvents, were separated the fractions 1, 2, 3, 4, 6 and 8 which were tested from point of view of their effect on cancerous cells development.

The possibility of a direct pursuit of the cytostatic effect the short time of investigation, the necessity of small amounts of substances, the positive correlation between the *in vitro* cytostatic action and *in vivo* antitumoral effect, registered in the case of many tested substances, justified the use — for this preliminary stage — of the "in vitro" testing system on HeLa cell cultures of human neoplastic origin in order to quantitatively estimate the cytostatic action of separated phenolic fractions (1), (3), (5), (13), (15), (16). The real quantitative evaluation of the inhibitory potential of the different fractions imposed, in some cases, the assessement of their solvents effect on cell cultures development.

The final inhibition of cell proteinosynthesis process at least of 50% represents the appreciation criterion of a substance as *in vitro* cytostatic and/or cytotoxic agent (5).

In relation to this criterion, the comparative analysis of the protein values which characterized the evolution of the control cultures, of the cultures treated with the phenolic fractions and of the cultures incubated with the corresponding solvents highlighted that :

— the fraction 1 — extracted in cyclohexane and redissolved either in cyclohexane (Fr. 1 a) or in ethyl acetate (Fr. 1 b) — probably containing latex, wax, fatty acids, fatty alcohols and terpenoids as phenolic structures, is lacked of cytostatic activity. The inhibition of the cultures incubated with Fr. 1 a or Fr. 1 b is not due to the chemical structures from this fraction, but it was due to their solvents (cyclohexane and ethyl acetate), which induced a strong cytotoxic effect (72.5% and 61.3% respectively) on cell cultures (see Fig. 1) ;

— the fraction 3 — extracted in ethanol and redissolved either in glycerol + water 1 : 1 (Fr. 3 a) or in water (Fr. 3 b) — probably containing flavonoids, stilbenes, proanthocyanidines, as phenolic structures and simple saccharides, was characterized by a significant cytostatic effect (about 65%). It is observed that the inhibitory action on the development of cell cultures during the whole experiment was not diminished by the solvent effect ;

— the fractions 2, 4, 6 and 8 represent the alkaline extracts (in NaOH) of a half from the residues obtained after succesive extractions of *Asclepias syriaca* leaves with cyclohexane, ethanol, ethyl ether, aceton and having a similar composition to that of the total polyphenolic preparations (PA_2, PA_2 III) from which there are missing totally or partially the chemical structures dissolved in the above solvents. They induced a significant inhibition of the HeLa cell cultures development, the values of the cytostatic

potential being in succession 56.6%, 61.2%, 58.1% and 73.8%, respectively.

It can be concluded that, excepting Fr. 1 represented by inactive cytostatic compounds, the others were characterized by a significant inhibitory potential, probably due not only to a phenolic structure but to many phenolic type substances from the studied fractions. This supposition is argued by the intensities of the induced cytostatic effect, which were however smaller than that one of the PA_2 III total polyphenolic preparation (83.6%).

At present, it is difficult to specify which phenolic components are responsible for the induced cytostatic effect, some categories of phenols finding again in the different fractions submitted to *in vitro* testing.

In can be appreciated that the methodology used for the obtaining of the phenolic structures did not lead to their selective extraction and to the separation of some structural homogenous fractions.

Therefore, for an accurate evaluation of the *in vitro* cytostatic action — as a selection preliminary step of an *in vivo* active cancerostatic agent — of the different phenolic structures it is necessary their selective separation and their identification by:

— the modification of the extracting solvents sequence;

— the assurance of certain optimum conditions to extraction;

— the diversification of the range of extracting agents for the selective separation of different phenolic structures;

— the exclusion of successive alkaline extractions and their replacement with a final alkaline extraction to render soluble the neextracted phenolic structures;

— the use of the chromatography in order to identify the separated phenolic structures.

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EVOLUTION OF CERTAIN STRCTURAL PARAMETERS OF ZOOPLANKTON UNDER EUTROPHICATION IMPACT IN THE ECOLOGICAL SEQUENCE OF LACUSTRIAN ECOSYSTEMS IN THE DANUBE DELTA

V. ZINEVICI and LAURA TEODORESCU

Three distinct, sequential formation stages are evident during the transition of the Danube Delta lacustrial ecosystems to the terrestrial type, i.e. : young lake stage ("ghiol"), old lake stage ("japşă") and temporary flooding stage. During the sequential formation of ecosystems the dynamics of structural parameters of zooplankton evidences the highest effects of the eutrophication impact which affects the early stage of the evolution process, while in the second and third stage its influence shows a gradual decrease.

The natural evolution of the Danube Delta lacustrian ecosystems shows, in the latest analysis, the gradual transition of the biotope and biocenosis from the aquatic to the terrestrial type . This process consists of three sequential formation stages, i.e., young lake ("ghiol"), old lake ("japşă") and temporary flooding stage.

Complex, anthropic reasons, as an increase in the nitrogen and phosphorus level in the Danube basin, induced, since **1970**, successive alterations in the trophic level of lacustrian systems from the Danube Delta such that all the stages ranging in between mesoeutrophy and hypertrophy were encountered during a decade. The newly created conditions favourized, in case of primary production of plant material, the development of the components which supported higher rates of nutrient recycling. As a result, a high proliferation of planktonic algae was noted since 1981 which induced "water blooming" determining a strong reduction — sometimes even a total elimination — of the submerged macrophytes. The structural changes affecting the primary producers result in evident changes of the environment heterogeneity as well as changes of the main environmental parameters.

Nevertheless the alteration degree generated by the above mentioned anthropic factor shows evident variations as a function of ecosystem formation stage. The decrease in the number of submerged macrophytes and phytoplankton proliferation evidenced peak values in case of rather deep waters (usually exceeding 1.6—1. 7m depth) characteristic of the first stage in the development of Danube Delta lacustrian ecosystems. With the second stage of sequential ecosystem formation, under the conditions of lower water levels (0.9—1.6m) the aquatic macrophytes become, in about 3—4 months, the main primary producers inhibiting phytoplankton proliferation. Nevertheless, at the end of macrophytes development period (which in the past lasted for a longer period) "water blooming" phenomena are often evident. The small water depth in the areas of lentic-terrestrial ecotones (usually 0.2—0.3m) which corresponds to the third stage of the sequential ecologic development, favourizes, independently of nutrients

evolution, the growth of aquatic and palustral macrophytes along all vegetation period, maintaining the phytoplankton evolution parameters at values comparable to those existing in the Danube Delta previously to the above mentioned anthropic impact.

The changes induced by eutrophication at the level of primary producers results in certain significant features which appear in the evolution of secondary consumers. A high number of data is available on the structural and functional evolution of zooplankton during the first stage of sequential formation of lacustrian ecosystems. According to these data, important reductions of the taxonomic structure (exceeding 53%) were evident during 1975—1987, accompanied by evident increases in numerical density (4.7 times), biomass (6.9 times) and productivity (4.8 times) as well as by the shortening of gravimetric recycling duration (with 15%) (2), (3). However, there is no available information on zooplankton evolution during the other stages of sequential ecologic development. The first studies on zooplankton dynamics as considered in the framework of the overall sequential formation of lacustrian ecosystems in the Danube Delta started in 1991. A part of the data regarding structural aspects are presented in this paper.

MATERIALS AND METHOD

The researches were performed during the particular hydrologic conditions of 1991, characterized by an absolute maximum and minimum during August and September-October, respectively, and by a secondary minimum recorded during May-June, i.e. in early spring. The researches concern young lake ("ghiol") zooplankton (the first stage of the sequential formation) (with Roşu, Matiţa and Merhei ecosystems), old lake ("japşă") zooplankton (the second stage) (with Porcu, Tătaru and Lopatna ecosystems), as well as zooplankton of the temporary flooding stage (the third stage) (with the following ecotones : Sf. Gheorghe river branch — Sacalin island, Erenciuc lake — Caraorman island and Merheiul Mic lake — Letea island). The average depth of the water layer during the period under research ranged between 1.6 — 2.2 m with the ecosystems of the first sequential development stage (minimum value — Merhei lake, maximum value — Roşu lake), between 1—1.6m with the ecosystems of the second stage (minimum value — Tătaru lake, maximum value-Porcu lake) and between 0.3—0.4m in ecotone zones (the third stage). The surface area of the young lakes ranges between 644 ha (Matiţa lake) and 1375 ha (Roşu lake), while that of the old lakes between 10 ha (Lopatna lake) and 100 ha (Porcu lake). The duration of the flooding period of ecotone zones was of about 90 days (July-August period). The particular hydrologic conditions of the above mentioned year, determined a reduction in the intensity of the "water blooming" phenomenon as compared to the previous years. Under these circumstances the phytoplankton biomass exceeded 4.6 times the "blooming" threshold of "ghiols" and 1.6 times the corresponding value noted with "japşa" (4) while in the ecotone zones it decreased reaching values located under the mentioned threshold (5) (According to M. Oltean (1), "water blooming threshold" corresponds to 5 mg/l fresh phytoplankton biomass). As regards the evolution of the space dynamics, the water macrophytes showed an opposite trend as compared to phytoplankton. Sampling was performed monthly using a Patals -Schindler sampling device. About 50 l of water were filtered with each collected sample.

RESULTS AND DISCUSSIONS

Taxonomic diversity of zooplankton reflects, in an important extent, the environmental heterogeneity, which in its turn shows the abundance degree of macrophytes. Under the ecologic conditions existing previously to eutrophication impact, with primary production based mainly on submerged macrophytes, the amplitude of the taxonomic composition characteristic to lacustrian ecosystems zooplankton during the first stages of sequential formation proved to be dependent, in a high extent on the surface area of the lake basin. Correlating these factors with the optimum dynamics of taxonomic diversity resulted in absolute maximum values of taxonomic diversity. This was evident with the taxonomic spectra of zooplankton from Iacub and Roşu lakes (undergoing the first stage of sequential formation) which evidenced, in 1975, a number of 189 and 164 components, respectively. From an ecological point of view, the lacustrian zooplankton consisted, during the above mentioned period, of a mixture of macrophytes — dependent forms euplanktonic and planktonophilic, as well as nektobenthic and neustonic forms. From the total number of 454 zooplanktonic components identified with ecosystems based on macrophytes as primary producers, only 63% were strictly specific while the remaining ones were also identified — after 1981 — in the structure of lacustrian systems with planktonic type primary producers, alongside with the zooplankton elements specific to both ecosystem types.

Submerged macrophytes disappeared from the large lakes (I-st stage) of the Delta, while still present — in a certain amount — within the small ones (II-nd stage) ; this resulted in a new distribution of biodiversity with the maximum values of taxonomic spectra recorded not in case of large lakes, but in case of the small ones. The dynamics of taxonomic diversity in the lakes undergoing the first stage of sequential ecologic development shows an evident decrease with time. For example, with the Roşu lake, the values recorded during 1975—1987 decreased from 164 components to 62.

During 1991, under the conditions of a slight recovery trand of macrophytes/phytoplankton ratio, the decrease in the taxonomic diversity was counteracted to a certain extent. A number of 103 components was identified with lakes which underwent the first sequential formation stage, while 130 components were present in those which underwent the second stage (3). The extreme values characterizing taxonomic diversity in case of the first category of ecosystems (64—79 components) are ecountered with the Roşu and Merhei ecosystems, while in case of the II-nd category (59—97 components), with the Porcu and Lopatna ecosystems. The situation is apparently paradoxical : the minimal taxonomic diversity is recorded with the largest lake under study (Roşu lake) while the maximum value of this parameter corresponds to the smallest lake (Lopatna). From

the total of 154 components identified with stages I and II of the ecosystems studied in 1991, only 16% are exclusively ecountered in the first and 34% in the second case, the remaining of 50% consisting of the forms which are common to both sequential development stages (table 1). Gradual increase, with time, in the proportion of forms, to be found in both stages (from 37% during 1981—1987 to 50% in 1991) reflects the trend of extension of eutrophiczation impact to the ecosystems undergoing the second stage. From the ecological view point the zooplankton specific to the first sequential formation stage evidenced a preponderance of euplaktonic and planktonophilic forms alongside with the macrophytes-dependent forms. The components belonging to the latest category are preponderant in the zooplankton of the second sequential development stage while those which are typical planktonic play only a complementary part. The structure of planktonic fauna with the three ecotone zones (stage III) comprises a total of 93 components (Table 1). Consecuently, as compared ot the values of diversity showed by the first two sequential formation stages, those characteristic to floodable zones are somewhat smaller. One of the explanations for this situation has to be looked for in the relatively low value of the ratio between

Table 1

Evolution of relative abundance of zooplanktonic components, numerically and gravimetrically (%) constant, during the sequential formation of lacustrian ecosystems in the Danube Delta under the impact of eutrophication

Zooplankton components	no %	Sequential formation stage — Ecosystem			
		Stage I („Ghiol")			
		Roşu	Matiţa	Merhei	$\bar{X}a$
Taxonomic spectrum	no	64	66	76	103
Constant forms	%	18.75	18.18	15.79	11.65
Numerically dominant forms	%	21.88	25.76	19.74	22.33
Gravimetrically dominant forms	%	21.88	22.73	18.42	15.38
		Stage II(„Japşă")			
		Tătaru	Porcu	Lopatna	$\bar{X}a$
Taxonomic spectrum	no	84	59	97	130
Constant forms	%	7.14	10.17	6.19	4.61
Numerically dominant forms	%	20.24	22.03	17.53	19.23
Gravimetrically dominant forms	%	20.24	22.03	12.37	16.92
		Stage III (Floodable zone; lentic-terrestrial ecotone)			
		Sf. Gheorghe river branch-Sacalin Island	Erenciuc lake-Caraorman Island	Merheiul Mic lake — Letea Island	$\bar{X}a$
Taxonomic spectrum	no	53	52	38	93
Constant forms	%	18.87	13.46	23.68	4.19
Numerically dominant forms	%	16.98	21.15	21.05	14.68
Gravimetrically dominant forms	%	20.75	17.31	23.68	12.58

the length of ecotone interference edge and the surface area of adjacent biotopes belonging to the two ecosystem types. Nevertheless the taxonomic analysis of zooplankton along the three ecotone zones revealed the occurrence of certain forms which were absent during the last 10—15 years in the plankton of lakes undergoing the first sequential formation stage; this phenomenon was evident to a lesser extent in case of the lakes undergoing the second stage (3). Biodiversity of lentic-terrestrial ecotone zooplankton is to a high extent the expression of an important environmental heterogeneity, generated, in its turn, by the high diversity and abundance of both aquatic and paludal macrophytes. The long duration of the flooding period in the dumped zones corresponding to terrestrial ecosystems as well as the "edging" phenomenon specific to ecotone zones enabled conservation of an important number of macrophytes-dependent components which are accompanied by neustonic and nektobenthic forms as well as by swamp and wetland components. It has however to be mentioned that the euplanktonic and planktonophilic components are weakly represented.

The comparative analysis of lacustrian zooplankton numerical density and biomass evidenced successive decreases along the three stages of sequential ecologic development: 402 — 297 — 198,1 number of individuals/l (Table 2); 5403.7 — 1772.7 — 1094.2 µg/l fresh matter (Table 3). A similar dynamics was noted with the constant components as considering their frequence in the overall taxonomic spectrum (i.e. 11.65 — 4.61 — — 4.19%) as well with the numerically dominant (22.33 — 19.23 — 14.68%) or gravimetrically dominant elements (15.38 — 16.92 — 12.58%) (Table 1), according to the increasing trend of the environmental heterogeneity degree.

As regarding certain features of the taxonomic spectrum as well as the values of certain structural parrameters, the zooplankton of floodable zones (sequential formation stage III) strongly reminds the planktonic consumers of lacustrian ecosystems during the first two stages characteristic of the period preceding the human impact of eutrophication. This illustrates the ability of ecotone zones to provide for the structural preservation of lacustrian zooplankton. The analysis of the structural parameters also evidenced that zooplankton is mostly affected by the eutrophication impact during the first stage of sequential ecologic development; the eutrophication effects show a gradual decrease during the II-nd and III-rd stage.

CONCLUSIONS

During the process of sequential ecologic development of the Danube Delta lacustrian ecosystems the dynamics of zooplankton structural parameters shows the highest anthropic impact of entrophication during the first stage of ecosystem transition (the "ghiol" stage), its effects decreasing subsequently in the following stages, i.e. II-nd (the "japşa" stage) and the III-rd (the periodical flooding stage).

— As regards certain features of the taxonomic structure, the zooplankton of floodable zones is, to a high extent, similar to the lacustrian zooplankton in the period preceding the onest of anthropic impact.

Table 2

Evolution of absolute (number of individuals/l) and relative (%) numerical abundance of zooplankton in the sequential ecologic development of lacustrian ecosystems in the Danube Delta under eutrophication impact

Componence	no %	Stage I ("Ghiols")				Stage II ("Japşă")				Stage III (Floodable zones)			
		Roşu	Matiţa	Merhei	X̄a	Tătaru	Porcu	Lopotna	X̄a	Sf. Gheorghe river branch — Sacalin Island	Erenciuc lake Cara-orman Island	Merheiul Mic lake — Letea Island	X̄a
C_1	no	372.2	419	366	385.7	252.3	377.9	233.1	287.8	150.5	300.2	137.6	196.1
Ciliata	%	3.55	27.09	18.83	16.90	4.04	23.07	6.73	13.10	0.07	1.63	2.91	1.53
Testacea	%	0.89	0.62	0.82	0.78	9.39	2.22	4.33	4.90	12.36	19.82	7.70	15.09
Ostracoda	%	0.11	—	—	0.02	0.12	0.32	—	0.17	1.59	—	—	0.41
Lamellibranchia	%	0.35	0.52	—	0.31	0.12	0.08	0.13	0.10	—	—	—	—
Rotifera	%	35.08	26.30	27.76	29.58	38.64	18.71	28.36	27.14	8.57	36.04	56.32	33.76
Cladocera	%	20.42	19.67	28.25	22.64	8.05	6.59	17.46	9.94	32.89	0.50	2.83	9.33
Copepoda	%	39.44	25.80	24.34	29.72	39.64	49.01	42.99	44.65	43.46	40.41	29.22	38.55
Cirripedia	%	0.16	—	—	0.05	—	—	—	—	—	—	—	—
Chironomidae	%	—	—	—	—	—	—	—	—	1.06	1.60	1.02	1.33
C_2	no	16.7	13.9	18.3	16.3	5.8	13.9	7.9	9.2	0.9	1.6	3.6	2
Ciliata	%	—	—	—	—	1.72	—	—	0.33	22.22	—	5.56	5.00
Rotifera	%	22.16	25.18	37.16	28.83	27.59	6.47	39.24	20.33	22.22	—	33.33	25.00
Copepoda	%	70.66	65.47	55.19	63.19	70.69	92.09	55.70	77.17	55.56	100.00	61.11	70.00
Cladocera	%	7.18	9.35	7.65	7.98	—	1.44	5.06	2.17	—	—	—	—
Total C_1+C_2	no	388.9	432.9	384.3	402	258.1	391.8	241	297	151.4	301.8	141.2	198.1

Table 3

Evolution of absolute (μg/l fresh matter) and relative (%) gravimetric abundance of zooplankton in the sequential ecologic development of lacustrian ecosystems in the Danube Delta under eutrophication impact

Componence	μg %	Stage I ("Ghiols")				Stage II ("Japşa")				Stage III (Floodable zones)			
		Roşu	Matiţa	Merhei	X̄a	Tătaru	Porcu	Lopatna	X̄a	Sf. Gheorghe river branch — Sacalin Island	Erenciuc lake Cara-orman Island	Merheiul Mic lake — Letea Island	X̄a
C_1	μg	3890.4	3153.1	7801.1	4948.2	1233	2057.8	1448	1579.6	2276.9	614.2	295.5	1062.2
Ciliata	%	0.02	0.30	0.05	0.10	0.25	0.44	0.12	0.29	0.01	0.26	0.20	0.08
Testacea	%	0.05	0.05	0.03	0.04	1.35	0.30	0.52	0.64	0.66	7.91	2.77	2.25
Ostracoda		0.27	—	—	0.07	0.63	1.41	—	0.78	2.64	—	—	1.88
Lamellibranchia	%	0.02	0.05	—	0.02	0.02	0.01	0.01	0.01	—	—	—	—
Rotifera	%	2.38	1.99	1.69	1.93	5.18	2.26	3.30	3.33	0.44	8.79	25.82	4.41
Cladocera	%	74.23	75.08	91.84	83.66	42.96	45.57	62.20	49.98	85.21	10.38	17.09	64.46
Copepoda	%	22.99	22.53	6.39	14.17	49.61	50.01	33.85	44.97	11.04	72.66	54.12	26.92
Cirripedia	%	0.04	—	—	0.01	—	—	—	—	—	—	—	—
C_2	μg	432.4	437.3	496.9	455.5	109.2	292.2	177.7	193.1	9.4	44.2	5	32
Ciliata	%	—	—	—	—	0.04	—	—	0.01	1.06	—	0.07	0.12
Rotifera	%	6.84	6.72	11.22	8.39	12.26	5.99	14.51	9.79	21.28	—	24 20	12.69
Copepoda	%	65.33	44.80	43.77	50.93	87.70	86.74	55.84	77.44	77.66	100.00	75.93	87.19
Cladocera	%	27.83	48.48	45.01	40.68	—	7.27	29.65	12.76	—	—	—	—
Total C_1+C_2	μg	4322.8	3590.4	8298	5403.7	1342.2	2350	1625.7	1772.7	2286.3	658.4	338	1094.2

— Considering the structural preservation ability of zooplankton, the Danube Delta floodable plain can play an important role in the ecological restoration of lacustrian type ecosystems.

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LE RÔLE ÉCOLOGIQUE DU BACTERIOPLANCTON DANS LE FONCTIONNEMENT DES ÉCOSYSTÈMES AQUATIQUES LACUSTRES DU DELTA DU DANUBE

2.2. GROUPEMENTS PHYSIOLOGIQUES DE MICROORGANISMES IMPLIQUÉS DANS LA RÉALISATION DU CIRCUIT DES PRINCIPAUX ÉLÉMENTS

DORINA NICOLESCU

Cette étude fait partie d'un cycle de travaux concernant l'apport de la microflore bactérienne planctonique dans la réalisation des principales fonctions des écosystèmes aquatiques lacustres du Delta du Danube (4) — le flux énergétique (5), la circulation de la matière et l'autoréglage. L'étude présente l'analyse des principaux groupements spécialisés de microorganismes qui interviennent dans certaines étapes du circuit biogéochimique des principaux éléments — C, N, S. Cette analyse vient de compléter la mise en évidence de l'implication de la microflore bactérienne dans la réalisation de la fonction de circulation de la matière par la capacité de dégradation et de synthèse bactérienne (6).

À l'encontre du caractère unidirectionnel du flux d'énergie par l'écosystème, les éléments chimiques autocréés dans les processus métaboliques présentent un mouvement cyclique, étant toujours réutilisés, passant toujours de la matière inorganique (le milieu abiotique) dans la matière vivante et inversement, en formant les soi-disant cycles biogéochimiques des éléments, constitués de deux circuits interconnectés — le circuit biologique et celui géochimique.

Il est connu que les cycles biogéochimiques des différents éléments se déroulent en spirale, intriqués entre eux (11), leur complexité et poids dépendant de la spécificité des écosystèmes. L'implication des microorganismes dans ces circuits est due (6) principalement à leur activité de dégradation des substances organiques (de nature autochtone ou allochtone) et aux synthèses cellulaires mais aussi à la capacité de mobiliser ces éléments des soi-disant « réservoirs » naturals (le fixage du N_2 atmosphérique, la solubilisation des phosphates insolubles, etc.).

Ainsi que nous l'avons déjà montré (6) « la plasticité » du métabolisme bactérien (11), autrement dit « l'induction enzymatique » (3) particularité qui résulte de l'habileté des microorganismes de former les enzymes nécessaires pour l'utilisation des différents nutrients présents dans le milieu, justifie la capacité métabolique élevée des microorganismes dans des conditions de milieu variables en temps des écosystèmes aquatiques deltaïques. D'autre part, l'existence des différenciations individuelles, sous le rapport du potentiel biochemique des bactéries (11) a conduit à l'apparition de certains types « specialisés » métaboliques qui peuvent utiliser un nombre limité seulement de nutrients — les groupements physiologiques de microorganismes impliqués dans certaines phases du circuit des principaux éléments des écosystèmes aquatiques du Delta du Danube.

2.2.1. Groupements physiologiques des microorganismes participants au circuit du carbone

En partant de ce que les bactéries représentent — auprès les microphytes et les macrophytes — l'une des principles composantes de la circulation du carbone dans les écosystèmes aquatiques deltaïques, on peut affirmer que tous les groupements spécialisés de microoprganismes participent (par la nécessité métabolique en carbone) au circuit de cet élément. La spécificité des écosystèmes aquatiques deltaïques, — le développement successif des différentes populations alguales, la grande quantité de macrophytes submersibles (en certaines périodes et zones) — nous a déterminé de considérer opportun d'analyser — entre les groupements spécialisés — les microorganismes qui produisent la dégradation de l'amidon, des pectines et des celluloses de la masse d'eau — substances hydrocarbonates issues à la suite de la mort de la composante végétale. Etant donné le poids élevé de ces hydrates de carbone dans les écosystèmes deltaïques, on peut dire que l'intervention des microorganismes dans leur dégradation représente une étape importante dans le remise en circulation des éléments bloqués dans ces structures chimiques complexes ainsi que dans l'évolution des écosystèmes aquatiques.

Les microorganismes qui dégradent l'amidon

L'amidon est un composant facilement dégradable grâce à l'amylase—enzyme extracellulaire, produite par plusieurs bactéries. Le grand nombre des microorganismes amylolithiques dans les écosystèmes aquatiques deltaïques montre la capacité élevée de dégradation de cet hydrate de carbone. Les déterminations effectuées dans les lacs Roşu, Puiu, Porcu, Iacub, Răducu au cours des années 1975—1979 ont mis en évidence des valeurs comprises entre 10^4—10^8 cell./l (tableau 1) par rapport au lac Matiţa-Merheiu en 1980 — de 10^4 cell./l (tableau 1). L'inexistence d'une dynamique saisonnère caractéristique à ce groupe d'organismes (tableau 1) est due aux permanentes sources productrices d'amidon (algues planctoniques présentant des successions populationnelles rapides, macrophytes submersibles ayant différents cycles de vie).

La connaissance de l'évolution des biocénoses des lacs analysés nous permet certaines observations : les limites les plus larges de variation de la valeur se trouvent dans le lac Iacub, qui présente une mosaïque de développements explosifs des cyanophicées et des développements de la vegetation submersible en été ainsi que l'existence des îles flottantes en certaines zones du lac — fourniseurs d'amidon dégradable à l'achèvement du cycle de végétation, les limites larges dans lesquelles évoluent les microorganismes amylolithiques du lac Porcu, aux amplitudes élevées eu 1977, correspondent au plus grand développement des macrophytes submersibles — *Ceratophyllum* et *Miriophyllum*, ainsi qu'au phénomène de mélange de l'eau à l'horizon de surface du sédiment (à cause des petites profondeurs).

Les microorganismes qui dégradent les pectines

Les pectines, composants insolubles dans l'eau, se trouvent en quantités significatives, différentes de point de vue chimique selon leur provenance. Elles sont attaquées par les bactéries aérobies, anaérobies et

Tableau 1

Groupments physiologiques des microorganismes impliqués dans le circuit du carbone des écosystèmes lacustres du Delta du Danube — dynamique saisonnière, $\bar{X}_g$ (no$\times 10^3$/l)

Saison	Printemps				Été				Automne			
Groupe / Lac	Amylolithiques	Pectinolithiques	*Cellulosolithiques* aérobies	*Cellulosolithiques* anaérobies	Amylolithiques	Pectinolithiques	*Cellulosolithiques* aérobies	*Cellulosolithiques* anaérobies	Amylolithiques	Pectinolithiques	*Cellulosolithiques* aérobies	*Cellulosolithiques* anaérobiés
						1976—1976						
Roşu	1.250	17.30	3.80	6,21	1.448	0.24	2,25	0,60	1.349	96,18	0,68	1,03
Puiu	1.490	14.43	5.65	8.23	1.440	1,20	1,65	1,47	4.088	13,59	1,59	2,48
Porcu	2.076	1.50	4.75	4.14	3.832	1,15	0,69	1,64	958	29.40	0,31	0,31
Iacub (1975)					726				100.000			
Răducu (1977)	5.250	1,25	15,75	3,50	4.000	250,00	5,50	4,50	5.037	48,73	9,00	39,50
						1980						
Matiţa	325	3.00	12,00	11,00	31	4,69	2,00	1,41	48	8,00	4,00	5,00
Merheiu	359	3,00	12,00	9,00	58	241,00	0,09	0,07	47	6,00	2,00	3,00
						1987						
Roşu	2.300	34,00	8,00	8,40								
Roşuleţ	1.732	154,00	5,50	12,00								

fungi, leur degradation se déroulant en plusieurs étapes, à partir de leur solubilisation (10). Les déterminations de la microflore bactérienne pectinolythique des lacs du Delta du Danube (tableau 1) relèvant des valeurs de 10^3-10^5 cell/l, bien inférieures à la microflore amylolithique. On observe, en général, un développement plus abondant en automne dans les lacs Roşu, Puiu et Răducu et en été dans les lacs Merheiu et Răducu.

La structure chimique différente des pectines en fonction du développement des composantes végétales des biocénoses aquatiques, leurs insolubilité sont des facteurs qui concourent tant à l'évolution de la courbe de développement de la microflore bactérienne pectinolythique dans la masse de l'eau qu'à leur entraînement dans le processus de sédimentation des résidus végétaux dans ces écosystèmes peu profonds, ayant comme résultat la dégradation ultérieure dans les sédiments.

Les microorganismes qui dégradent la cellulose

La cellulose est un des plus complexes et stables hydrocarbonates issus en grandes quantités des restes végétaux des biocénoses des écosystèmes aquatiques deltaïques ; elle est attaquée seulement par les microorganismes cellulosolythiques spécialisés dans l'utilisation de la cellulase comme unique source de carbone. L'intensité du processus de dégradation aérobie et anaérobie de la cellulase dans la masse de l'eau est réduite ; elle lieu de préférence en sédiments pendant longtemps. Nos recherches ont mis en évidence une microflore cellulosolythique aérobie et anaérobie (tableau 1) dont les limites montent à 10^3-10^4 cell/l, dominante le printemps.

Les tests effectués en 1987 sur les mêmes groupes de microorganismes (tableau 1) relèvent des valeurs numérique élevées, ce que suppose l'accroissement du substrat dégradable dans le contexte de l'évolution des écosystèmes aquatiques deltaïques.

L'analyse quantitative de ces trois groupes physiologiques de microorganismes (spécialisés du point de vue de leur potentiel biochimique, y compris métabolique) illustre certains aspects de détail de l'intervention des microorganismes planctoniques dans la circulation de la matierè des écosystèmes aquatiques lacustres du Delta du Danube, à savoir : le carbone bloqué à la suite du processus de photosynthèse dans des composants comme l'amidon, les pectines, les cellulases, dans l'immense quantité de microphytes et macrophytes, est remis en circulation grâce aussi à la microfore bactérienne planctonique (facilement dégradable), le niveau le plus élevé de dégradation des pectines et des cellulases se déroulant dans les sédiments.

2.2.2. Groupements physiologiques de microorganismes participants au circuit del'azote

Golterman, 1975 (3) et Botnariuc, 1982 (1) ont montré que la recirculation de l'azote dans les écosystèmes aquatiques est déterminée par le circuit algual et bactérien, d'environ 10—20 fois par an. Toutes les composantes biocénotiques y participent par leur métabolisme, mais la capa-

cité d'utiliser et de fixer en constituents cellulaires l'azote en differentes formes d'oxydation existantes dans la masse de l'eau des écosystèmes aquatiques deltaïques revient aux algues et aux bactéries.

Dans les écosystèmes aquatiques, à l'encontre des autres éléments, l'azote se trouve dans divers stades d'oxydation, la microflore bactérienne déterminant en grande partie l'interconversion des différentes formes de cet élément. Selon Golterman, 1972 (3) : Le degré d'oydation du

N : $NO_3^- \xrightarrow{2e} NO_2^- \xrightarrow{3e} N \xrightarrow{3e} NH_3 \rightarrow$ N organique. La charge él. de l'atome

N : +5 +3 0 −3 −3.

Le cycle biogéochimique de l'azote connu (3, 8) met en valeur l'intervention complexe des microorganismes par groupements physiologiques spécialisés dans chaque étape de transformation entre les formes oxydée et réduite, apparemment comme un circuit exclusivement bactérien.

Dans les écosystèmes aquatiques deltaïques, la grande quantité de micro- et macrophytes est déterminée dans le circuit de l'azote par deux aspects : les nécesités accrues d'azote assimilable (NO_3^-, NO_2^-, NH_3) et les énormes quantités de matière organique provenue de la succession rapide des populations alguales et l'achèvement du cycle de vie des macrophytes qui s'imposent à être dégradées pour refaire les formes assimilables.

La minéralisation de l'azote organique issu à la suite de la chute des plantes, de la mort des animaux ou bien des sécrétions ou excrétion des algues se réalise par des groupements de microorganismes spécialisés pour chaque étape.

La dégradation des protéines complexes se produit, généralement, en dehors des cellules, à l'aide des enzymes protéolithiques (bien que les microorganismes continnent aussi des endoenzymes protéolithiques) jusqu'aux formes qui peuvent entrer dans la cellule où elles sont utilisées ou dégradées en produits utiles (11). La dégradation des protéines suit le schéma suivant : protéines → peptides → aminoacides.

Nous avons surpris cette étape de dégradation des protéines à l'aide des recherches portées sur *le groupment physiologique des protéolithique* qui comprend des bactéries strictement aérobies, facultativement anaérobies et strictement anaérobies. Les recherches effectuées dans les écosystèmes lacustres deltaïques (tableau 2) au cours des années 1975—1979, revèlent des valeurs de 10^2-10^6/l, plus élevées en automne, atteignant un maximum dans le lac Răducu. En même temps, les recherches effectuées dans le lac Roşu en 1987, metten en évidence une augmentation de deux ordres de grandeur par rapport à la période 1975—1979. Il faut mentionner que la formation des enzymes protéolithiques des bactéries est régressée par la présence dans le milieu d'un hydrolisé de protéines ou des aminoacides, donc par le produit même de leur activité.

Les aminoacides et les peptides ayant la masse moléculaire réduite, résultés du processus de protéolise, sont activement transposés dans les cellules microbiennes et métabolisés en poursuivant deux processus : la désamination et la décarboxylation, certains microorganismes ayant la capacité d'utiliser les deux possibilités à l'aide d'un mécanisme spécifique de régler le pH cellulaire. Dans le circuit de l'azote l'important c'est le processus de désamination avec formation de NH_3 ; le produit de la désamination est un composé qui peut être introduit directement dans l'une des

Tableau 2

Groupement physiologiques des microorganismes impliqués dans le circuit de l'azote des écosystèmes lacustres du Delta du Danube — dynamique saisonnière, $\bar{X}_g$ (no × 10⁶/1)

Saison	Printemps				Eté				Automne			
Groupe* Lac	1	2	3	4	1	2	3	4	1	2	3	4
						1975—1979						
Roșu	0,02	5,10	6,61	0,003	0,12	2,57	7,29	0,18	0,18	10,46	25,90	0,0380
Puiu	0,02	1,42	2,99	0,030	0,05	8,83	2,00	0,06	0,09	0,99	4,04	0,0002
Porcu	0,03	7,22	0,06	0,110	0,01	4,09	0,05	0,04	0,50	16,95	54,47	0,0100
Iacub (1975)	—	0,0003			0,0003	68,65	30,82		0,0002	500,00	89,78	
Răducu (1977)	0,18	34,50	275	1,600	1,84	38,08	6,00	0,04	2,50	50,00	50,00	0,2500
								1980				
Matița		51.645	77,522	0,040		2.322.000	23.199	0,11		20.000.000		2,5000
Merheiu		858	239	0,060		8	790	0,17		292		2,5000
								1987				
Roșu	6,98	3,27	6,13	0,020								
Roșuleț	1,69	0,98	4,20	0,050								

X = moyenne géométrique
*: 1 = Protéolithiques; 2 = Ammonificateurs; 3 = Dénitrificateurs; 4 = Fixateurs de N_2 anaérobies

voies du métabolisme énergétique ou un composé qui peut être facilement métabolisé pour produire une source potentielle d'énergie, NH_3 pouvant être utilisé dans la synthèse d'autres substance azotées. Le groupement physiologique entraîné, dans ce processus est celui *des ammonificateurs* mis en évidence dans les écosystèmes lacustres (tableau 2) ayant le poids le plus élevé de tousles groupements physiologiques spécialisés de microorganismes. On a relevé des valeurs comprises entre 10^2—10^8 dans la période 1975—1976 et 10^6—10^{12} en 1980. La présence des valeurs accrues généralement en été—automme et surtout dans le lac Matița en 1980, atteste, comme nous l'avons déjà montré (6) la présence, au cours de la « floraison » alguale et surtout au cours de leur sénescence, de certains produits facilement dégradables (même aminoacides) dans la masse de l'eau, attaqués par la microflore bactérienne adéquate.

Il faut mentionner qu'on a testé, auprès ces groupes de microorganismes qui participent à la minéralisation de l'azote organique dans les lacs du Delta du Danube, *le groupe des microorganismes uréolithiques*, ayant une présence de 10^3—10^9/l. En tout cas, la dégradation de l'urée (provenue spécialement des excrétions) par les microorganismes, représente une étape importante dans la recirculation des immenses quantités d'azote (9).

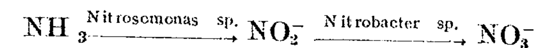
Golterman, 1975, montre que sur la quantité totale d'azote particulé, 50—75% seulement en est convertie en NH_3 par les processus métaboliques bactériennes, le reste étant consommé pour les nécessités de constitution des propres protéines bactériennes, pour les demandes énergétiques bactériennes, d'une part et, de l'autre, ce qui n'a pas pu être hydrolisé ou utilisé par les bactéries, se sédimente avec les résidus alguaux.

La présence des groupements physiologiques de microorganismes qui participent à la minéralisation de l'azote organique dans la masse de l'eau de l'écosystème lacustre deltaïque, aux densités numériques élévées pouve l'existance de la capacité de recirculation de l'azote inclus dans les composantes des biocénoses de ces écosystèmes, jusqu'à l'une des formes réduites minéralisées —NH_3, très assimilable par une grande partie des macrophytes.

En même temps, il faut rappeler que par décarboxylation, l'une des principales voies (à côté de la désamination) de la metabolisation des aminoacides par des microorganismes, se met en évidence l'interconnection du cycle biogéochimiques de l'azote et du cycle biogéochimiques du carbone, qui constitue l'un des processus par lesquels s'élimine le CO_2.

L'oxydations des composés de l'azote

Le processus biologiques d'oxydation de l'ammoniaque ou des autres formes réduites de l'azote résultées au cours du processus d'ammonification jusqu'aux nitrates, la forme la plus oxydée, a lieu à deux reprises à cause des groupements physiologiques distincts de microorganismes. Dès 1890, Winogradski a isolé deux bactéries du g. *Nitrosomonas* et *Nitrobacter*, qui réalisent les deux stades d'oxydation de l'azote, bacteréries obligatoirement chémolitotrophes, ne pouvant pas être cultivées dans des milieux qui continnent des substances organiques.



$$NH_3 \xrightarrow{\text{Nitrosomonas sp.}} NO_2^- \xrightarrow{\text{Nitrobacter sp.}} NO_3^-$$

Chaque stade de ce processus produit l'énergie utilisée par les bactéries pour réduire le CO_2.

Le processus d'oydation des formes réduites de l'azote a été mis en évidence dans les écosystèmes lacustres du Delta du Danube, par *nitrite-bactéries* (nitrosobactéries) et *nitrate-bactéries* (nitrobactéries) responsables des deux phases du processus. Golterman, 1975, (3) montre que la température réduite et la présence des substances organiques mènent à un taux diminué de l'oxydation ; en même temps, Daubner et Ritter, 1979 (1a), ont relevé la dépendance de l'activité nitrificatrice de la concentration d'O_2 dissout du milieu. L'identitication des nitrite- et nitrate -bactéries dans les lacs deltaïques a été très difficile : elles ont été maises en évidence en quantités décélables en cultures d'une manière sporadique, après une prolongée période d'incubation. Ainsi, a-t-on signalé les nitrite-bactéries, en 1975, dans le lac Iacub — entre les limites de $10^5/-10^7/1$ et en 1977 — dans le lac Răducu ($10^4-10^5/1$), écosystèmes dans lesqels le processus même d'ammonification a été plus élevé (tableau 2). La phase de nitratation du processus a été signalée seulement en 1977 dans le lac Puiu et la mare de Porcu aux densités bien inferieures ($0-10^4/1$) en certaines périodes (en été). G. Zarnea, 1984 (11), montre que le développement des bactéries nitrificatrices nécessite une consommation accrue d'énergie pour la réalisation d'un « transfert inverse d'électrons », ce qui explique pourquoi l'accroissement de *Nitrobacter* nécesite l'oxydation d'une grande quantité de nitrites et pourquoi cet accroissement est lent.

En même temps, les écosystème lacustres deltaïques qui se trouvent dans un stade avancé d'eutrophie abondent en substances organiques ayant un rôle inhibiteur, ce qui justifie le faible développement des bactéries nitrificatrices autotrophes.

Gode et Overbeck, 1972 (2), ont supposé et recherché la posibilité de réaliser cette étape d'interconversion des formes réduites en formes oxydées de l'azote, dans les eaux eutrophes, par une microflore bactérienne hétérotrophe ; les recherches comparées dans le lac Plusse ont mis en evidence que l'oxydation de l'ammoniaque est due presque entièrement aux bactéries hétérotrophes.

Les épreuves que nous avons effectuées dans le lac Puiu, en 1979, ont montré qu'il y avait une *nitrification hétérotrophe*, le processus étant plus intense aux moments où la microflore totale hétérotrophe était plus abodante et la nitrification autotrophe presque inexistante. (7).

Le processus de nitrification a une signification majeure dans la biologie globale des écosystèmes aquatiques, en réalisant la forme la plus assimilable de l'azote par les micro-et macrophytes- NO_3^-. A cause des grands nécessités d'azote dans les synthèses cellulaires, les algues utilisent quand même l'azote sous la forme — NH_3 (d'une manière préférentielle). Ce fait est attribué à l'inhibation de l'enzyme nitrate réductase par le NH_3 présent dans le milieu (3).

La réduction des nitrates par des microorganismes se réalise outre la réduction assimilatrice par laquelle tout microorganisme capable d'utiliser NO_3^- en tant que source d'azote produit la réduction des nitrites à NH_3 nécessaire à la biosynthèse des constituants cellulaires par un processus de désassimilation aussi, de réduction de l'un ou de tous les deux produits oxydés de l'azote aux oxydes gazeux (NO ou N_2O) qui peuvent être, eux aussi, réduits jusqu'à l'azote gazeux (N_2). Le processus est connu sous le nom de dénitrification, effectué exclusivement par les bactéries qui composent un groupe biochimique et taxonomique très hétérogène — *le groupe des dénitrificateurs*. Dans ce processus, le nitrate ou les produits de sa réduction servent en tant qu'accepteurs d'électrons pour l'oxydation de certains composants , généralement organiques et pour la production d'énergie. La réduction des nitrates peut se dérouler jusqu'à la production et l'élimination de $NH_3(NO_3^- \rightarrow NO_2^- \rightarrow NH_3)$, tenant compte des conditions existantes dans l'écosystème. La dénitrification est un processus multienzymatique, sensible à la présence de l'oxygène qui même à la répression de la synthèse des enzymes dénificatrices. Il a été long temps considéré comme processus qui n'a pas lieu dans les eux bien dans les eaux bien oxygenées. Jannach et Pritchard , 1972, ont démontré que le processus de dénitrification se produit aussi dans les eaux ayant un contenu elevé de O_2 dissout, mais avec beaucoup de suspensions, dues peut-être à une zone microanaérobie qui se forme autour des particules en suspension. C'est la situation existante dans les lacs du Delta du Danube qui ont, pendant la plupart de l'année, une quantité maximale d'oxygène dans la masse de l'eau (due au fort développement de la micro- et de la macrovégétation) et abondent en suspensions. Les bactéries dénitrificatrices, anaérobies et, pour la plupart, hétérotrophes, utilisent le nitrate pour l'oxydation complète de certains composés organiques à CO_2 et eau. Elles ont été identifiées en grandes quantités dans les écosystèmes lacustres deltaïques — entre les limites de $10^4-10^{11}/1$, prédominantes au cours des années 1775—1979 dans les lacs Iacub et Răducu et la mare Porcu, le maximum étant atteint dans le lac Matița en 1980 (tableau 2).

La dénitrification est un processus biologique particulièrement important dans les lacs du Delta du Danube par plusieurs aspects :

— une activité élevée de denitrification réduit les quantités de NO_3^- jusqu'à N_2, inaccessible aux microphytes et aux macrophytes ;

— la production de N_2 et même de NH_3 mène à la parte d'importantes quantités d'azote de l'écosystème bien qu'une partie de NH_3 puisse revenir dans la circuit propre de l'écosystème prélevé par d'autres organismes ;

— l'éventuelle temporisation des étapes de la nitrification, de répression de la synthèse des enzymes nitritereductases (à cause de la modification des conditions du milieu) pourrait créer des conditions défavorables aux biocénoses par l'existence d'une quantité de NO_2^- ayant des effects toxiques.

Grâce aux mécanismes d'autoréglage les écosystèmes tendent vers le redressement des réserves d'azote nécessaires au développement des biocénoses, par le processus de fixation biologique du N_2 atmosphérique, réalisée par un groupe restreint d'organismes, ayant un système enzymatique spécifique — la nitrogénase. Par rapport aux conditions spécifiques des écosystèmes aquatiques deltaïques, nos recherches ont poursuivi la mise en évidence des bactéries fixatrices de N_2 atmosphérique, hétérotrophes libres aérobies et anaerobies. G. Zarnea, 1984 (11), montre que la nitrogénase, même celle provenue des bactéries anaérobies, est rapidement et irréversiblement inactivée par la présence de l'oxygène, mais les bactéries aérobies ont dévelopé des mécanismes, des adaptations et des stratagèmes anatomiques et physiologiques pour éviter l'accès de l'O_2.

La présence des bactéries fixatrices de N_2 atmosphérique dans la masse de l'eau a été signalée, pour celles aérobies en 1975—1976 aux valeurs réduites (10^2—10^4/1) pendant que les bactéries anaérobies ont été identifiées au cours de l'entière période de recherche aux valeurs comprises entre 10^3—10^6/1 (tableau 2). Il faut mentionner que la réduction enzymatique du N_2 atmosphérique à NH_3 est un processus necessitant une grande quantité d'énergie pour rompre la triple liaison du N_2 ($N \equiv N$) ayant un degré élevé de stabilité ; elle est toujours accompagnée d'une hydrogénase capable d'augmenter l'efficience du fixage du N_2 (11). La synthèse de la nitrogénase se trouve sous le contrôle rigoureux du NH_3 au rôle repressiff, de sorte que les microorganismes ne puissent fixer le N_2 atmosphérique que lorsque l'azote fixé manque du milieu, et c'est là peut-être la cause des grandes besoins d'énergie.

Par la diversité des groupement physiologiques de microorganismes présents dans chaque étape d'interconversion des différentes formes de l'azote, le circuit de cet élément dans les écosystèmes aquatiques peut être considéré comme un circuit bactérien. Ce n'est qu'en apparence, parce que seules les étapes de transformation sont propres aux bactéries, pendant que la synthèse des protéines du protoplasme cellulaire appartient pour la plupart aux plantes et aux animaux (tout comme aux bactéries) et le processus de fixation du N_2 atmosphérique, de compensation des pertes d'azote dans l'écosystème, est dû dans une petite mesure aux bactéries, dans les écosystèmes aquatiques deltaïques les algues bleues-vertes (les cyanobactéries) accomplissant cette fonction avec bien plus d'efficacité.

2.2.3. Groupements physiologique de microorganismes participants au circuit du soufre

Constituant de quelques aminoacides importants et de certaines substances protéiques et vitamines, le soufre est un élément de base de la composante biotique des écosystèmes aquatiques, son circuit étant un exemple spécifique pour l'interconnection aux circuits des autres éléments (N, P, Fe). En même temps, le soufre et ses composés deviennent toxiques pour les plantes et les animaux lorsque sa quantité dans le milieu est en excès. Ayant un caractère mixte — gazeux et sédimentaire, le circuit biologique du soufre se déroule dans les écosystèmes aquatiques fortement dépendant du circuit hydrologique et des processus physico-chimiques, des échanges avec les sédiment et l'atmosphère. Le rôle clé dans le déroulement du circuit biogéochimique du soufre revient aux microorganismes, autant par les processus de dégradation des substances organiques à soufre que par les transformations produites entre les formes oxydées et celles réduites de cet élément (1).

Compte tenu des conditions des lacs du Delta du Danube, caractérisées par grandes quantités de substances organiques, conditions différentes d'oxygénation et infiltrations d'eau marine en diverses zones, nous avons considéré nécessaire de mettre en évidence quelques groupements spécifiques de microorganismes impliqués dans le circuit de cet élément.

La dégradation des substances protéiques avec prouction de H_2S, déroulée dans des conditions réductrices, est due à un groupe de microorganismes non spécifiques, important dans le remise en circulation du

soufre contenu dans les substances organiques complexes, mais aussi en tant qu'épreuve de l'interconnexion des circuits de l'azote et du soufre. En général, dans les lacs du Delta du Danube, ce processus se produit là où il y a des conditions de déficit d'oxygène, dans les zones d'îles flottantes, et, bien entendu, dans les conditions où il y avait des formes assimilables de soufre (sulfates) qui ont été incorporées dans les composantes cellulaires. Les valeurs prélevées du lac Roșu sont dues à l'apport de la zone du voisinage des îles flottantes du lac Roșuleț et quelque fois à l'apport de l'eau de mer riche en sulfates. Les maxima energistrés en 1980 dans les lacs Matița et Merheiu (tableau 3), au cours de l'automne et de l'été sont dus aux mêmes facteurs de circulation de l'eau avec un chargement organique du côte des îles flottantes.

Les sulfato réducteurs se développent aussi dans des conditions réductrices dans lesquelles les sulfates sont réduits aux formes moins oxydées ou même à H_2S.

En général, le processus de sulfatoréduction a lieu dans les sédiments, mais il se développe aussi dans la masse de l'eau dans les zones du voisinage des îles flottantes ou dans les horizons de profondeur lorsqu'ils ne sont pas bien oxygénés. Ce groupe est le mieux représenté dans le lac Iacub (1975) en été-automne, dans le lac Răducu en été (1977) et dans le lac Matița au printemps, périodes pendant lesquelles les aux eaux situées sous les îles flottantes ont été entraînées (tableau 3).

Tableau 3

Groupements physiologiques de microorganismes impliqués dans le circuit du soufre dans les écosystèmes lacustres du Delta du Danube — dynamique saisonnière, $\bar{X}g$ (no. × $10^3/l$)

Lac	Saison / Groupe	Printemps 1	Printemps 2	Été 1	Été 2	Automne 1	Automne 2
	1075—1979						
Roșu				80,86	2,15	45,00	8,12
Puiu				9,75	2,61	1,50	1,10
Porcu				108,65	5,52	4,50	1,59
Iacub (1975)					98,00		80,00
Răducu (1977)					18,30		2,50
	— 1980 —						
Matița		150.000,00	21,00	0,00	7,78	170,00	4,00
Merheiu		1.230.000,00	7,00	0,07	8,12	250,00	8,00
	— 1987 —						
Roșu		1,10	3,40				
Roșuleț		0,90	0,00				

1 = décomposants avec librération de H_2S
2 = sulfatoréducteurs

Il faut mentionner que dans le Delta du Danube, le produit de l'activité métabolique de ces deux groupements de microorganismes — H_2S, s'accumule dans toutes les zones des îles flottantes ou dans les zones où

se produisent des dégradations massives faute d'oxygène et il entre automatiquement dans la circulation des eaux, lorsque, aux niveaux réduits, l'eau sort de sous les îles flottantes dans le système de ruisseaux et canaux. Le H_2S entre de cette manière dans le circuit hydrologique (N. Botnariuc, 1982) ; il peut être soumis aux oxydations chimiques ou biologiques ou peut être sédimenté en formes réduites du soufre.

Le processus de *sulfoxydation* dû aux différentes espèces de *Thiobacillus* se déroule dans les endroits où il y a une source permanente de H_2S et O_2 (ou NO_3^-) parce que le H_2S n'est pas stable et il est oxydé par voie chimique. Les microorganismes qui composent ce groupe sont chémolithotrophes, spécialisés dans l'oxydation d'une large gamme de substrats réduits jusqu'à S^0 élémentaire ou/et formes oxydées, comme sulfates, sulfites. Les épreuves que nous avons effectuées dans les lacs du Delta du Danube ont relevé la présence des bactéries sulfoxydantes du g. *Thiobacillus* de l'ordre $10^1-10^5/1$, surtout dans les zones potentielles productrices de H_2S.

L'analyse quantitative numérique des différents groupes spécialisés de microorganismes participants aux différentes étapes du circuit des principaux éléments des lacs du Delta du Danube, met en évidence un tableau d'ensemble de l'implication des microorganismes dans les processus de dégradation et de transformation des différentes formes chimiques dans lesquelles se trouve l'élément dans les écosystèmes. La comparaison d'un groupement physiologique dans différents lacs étudiés par la densité numérique qui le compose relève l'activité métabolique par rapport aux conditions spécifiques de chaque écosystème ; une comparaison du poids numérique entre différents groupements participants au circuit du même élément ou de différents éléments n'est pas édificate parce que le développement d'une groupement dépend de sa physiologie et sa biochimie, de la facilité dans l'utilisation du substrat, des facteurs répressifs sur leur potentiel enzymatique, des nécessités énergétigues, etc.

Il faut mentionner que dans la réalisation du circuit des éléments dans les écosystèmes, le développment des groupements physiologiques spécialisés est soumis à la loi de la succession écologique, le développement des groupes créant dans les écosystèmes, par le métabolisme cellulaire, des sources de nutrients nécessaires à d'autres groupes.

L'intervention des groupements physiologiques spécialisés dans l'interconversion des différentes formes sous lesquelles se trouvent les éléments dans les écosystèmes aquatiques deltaïques détermine l'existence des formes assimilables pour les composantes biocénotiques et assure l'équilibre entre les conditions réductrices et celles oxydatives du milieu.

Il est d'autant plus évident que dans les recherghes intégrées, dans l'esprit de l'analyse systémique, la simple détermination du nombre des microorganismes appartenant aux différents groupements physiologiques impliqués dans le circuit biogéochimique des éléments est induffisante ; il est opportun de quantifier les di ifférentes étapes de l'interconversion des diverses formes chimiques dans l'appréciation de la fonctionnalité de l'écosystème dans les conditions de l'impact anthropique (7).

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