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COMUNITĂŢI ALGALE DIN MLAŞTINILE DE TURBĂ DE LA PEŞTEANA ŞI BĂGĂU

LAURA MOMEU* şi LEONTIN ŞTEFAN PĂTERFI**

SUMMARY. — Algal Communities of the Peşteana and Băgău Peat Bogs. The present paper deals with the structure of algal communities inhabiting the peat bogs situated near Peşteana (The Haţeg Depression) and Băgău (The Târnava Valley). Our investigation revealed that the bog located near Peşteana, with a eutrophic algal flora at the end of the 19th century, became in the last 100 years a mesotrophic peat bog with typical oligotrophic central enclaves.

The Băgău peat bog exhibits a very peculiar algal flora with a sphagnophilous community consisting only of few species.

According to community structure, both peat bogs are under high anthropic stress, exhibiting a large number of coenoxene forms.

Mlaştinile pe care dorim să le abordăm din punctul de vedere al comunităţilor algale sunt situate în etajul pădurilor de foioase, la altitudini cuprinse între 440 şi 480 m s.m. Fiecare mlaştină prezintă o serie de caractere care o individualizează, în funcţie de modul de formare şi de factorii de mediu care au acţionat în timp asupra celor două lacuri existente iniţial, inclusiv influenţele antropice.

Mlaştina de la Peşteana este situată în Depresiunea Haţegului, iar cea cunoscută sub denumirea de „Tăul fără fund" de la Băgău, în Depresiunea Târnavelor. „Tăul fără fund" se află la 7 km nord-est de Aiud, respectiv la 2 km nord-nord-est de satul Băgău. S-a format prin alunecări de teren în stratele sarmatice, la o altitudine de 440 m. Această mlaştină a fost semnalată de C s a t ó în 1896 [1], flora fiind studiată de către P o p în anii 1930 [10, 11].

Algele nu au fost studiate până în prezent, fapt ce ne-a determinat să abordăm acest subiect.

Cu ocazia cercetărilor pe care le-am efectuat în lunile iulie şi august 1991, am găsit mlaştina într-o fază avansată de colmatare. Suprafaţa acelui „lac tulbure" care ocupa o treime din totalul mlaştinii [10, 11], s-a redus considerabil. În ultimii ani, apa lacului a fost folosită pentru adăparea vitelor din satul amintit, ceea ce a dus la degradarea continuă a întregului ecosistem. În porţiunea estică, înmlăştinată, se pot distinge uşor cele două părţi descrise de P o p [12], respectiv cea eutrofă periferică şi cea mezo-oligotrofă centrală. Covorul vegetal a rămas practic neschimbat sub aspectul compoziţiei floristice, fiind regăsite majoritatea plantelor semnalate. Menţionăm însă că stratul de turbă din complexul central oligotrof atinge peste 5 m grosime, faţă de numai 3,5 m cât avea acum 60 de ani [10, 11]. Stratul muscinal este

* *Institutul de Cercetări Biologice, 3400 Cluj-Napoca, România*
** *Universitatea Babeş-Bolyai, Catedra de biologie vegetală, 3400 Cluj-Napoca, România*

format doar din 3 specii: *Sphagnum recurvum, S. magellanicum* şi *Polytrichum strictum.* Stratul ierbos este dominat de *Eriophorum vaginatum, Molinia coerulea şi Droşera rotundifolia,* specii semnalate şi de Pop [10, 11]. Remarcăm numărul redus de plante, precum şi uniformitatea accentuată care se manifestă la nivelul vegetaţiei.

Mlaştina de la Peşteana este situată la nord-vest de comuna Peşteana, la o altitudine de 480 m s.m. Schaardschmidt [14] semnalează în 1880 un lac („La lac"), format în căldarea unui deal. Încă la acea dată, în mijlocul lacului începuse să se înfiripe o insulă mlăştinoasă, pe care apăruseră primii mesteceni. Acelaşi autor colectează şi identifică primele alge (Tabel 2). În 1971, Pop şi colab. [13] menţionează că mlaştina „este în stadiu de colmatare recentă prin sfagnetizarea unui lac pleistocen format prin profuziune mecanică în urma scurgerilor superficiale îndreptate în direcţii concentrice spre cuveta situată la marginea platoului, platou al ultimei culmi a Masivului Poiana Ruscă.

În vara anului 1968, unul dintre noi (L. S. Péterfi), colectează un bogat material algologic din mlaştina de la Peşteana, material pe care îl vom folosi în lucrarea de faţă, alături de cel recoltat de noi. La acea dată, suprafaţa lacului era aproape complet ocupată de un sfagnet mezotrof compact, care mai păstra, totuşi, câteva ochiuri de apă în zona centrală şi avea un lagg bine individualizat. Conform cercetărilor lui Pop şi colab. [13], sfagnetul este, de fapt, un plaur alcătuit dintr-un strat de turbă recentă de 1,5 m grosime în zona sa centrală, care pluteşte pe o pânză de apă de 2 m. Sub pânza de apă se găseşte un strat de 1,2 m turbă fini-glaciară, care se continuă cu un mâl necrotic „Gyttja" de 40 cm, aşezat la rândul său pe sedimente compacte de argile nisipoase, dispuse la sfârşitul primei glaciaţiuni.

Cu ocazia cercetărilor de teren pe care le-am efectuat în iulie şi august 1991, am găsit mlaştina într-o fază şi mai avansată de colmatare, suprafaţa lacului semnalat de Schaarschmidt la sfârşitul secolului trecut [14] era total înmlăştinită, zona de lagg restrângându-se considerabil doar la o mică porţiune, în partea de vest a acesteia.

Mlaştina este deosebit de umedă, cu o vegetaţie bine închegată, reprezentată de asociaţia *Carici rostratae-Sphagnetum recurvi* Zóly., invadată de mesteacăn şi arin [13]. Păşunatul intensiv care se practică în zonă a influenţat, aşa după cum vom vedea în continuare, şi flora algală existentă.

Material şi metodă. Pentru stabilirea structurii comunităţilor algale sfagnofile din cele două mlaştini prezentate mai sus (Tabel 1) am folosit mai multe eşantioane, colectate după cum urmează:

mlaştina de la Peşteana — probe colectate în iulie 1968

1. plancton din zona de lagg;
2. biodermă de pe frunze din lagg;
3. bentos din marginea internă a lagg-ului;
4. sfagnoplancton din zona centrală a mlaştinii;
5. metafiton de *Utricularia;*

6. mase gelatinoase din adânciturile apătoase ale mlaștinii;
7. mase gelatinoase din adânciturile situate în aria centrală a mlaștinii.

mlaștina de la Pesteana — probe colectate în august 1991

8. plancton din zona de lagg;
9. mase gelatinoase din adânciturile apătoase ale zonei centrale.

„Tăul fără fund" de la Băgău — probe colectate în iulie 1991

10. mase gelatinoase din adânciturile apătoase ale mlaștinii în zona periferică;
11. mase gelatinoase din adânciturile apătoase ale mlaștinii în zona centrală.

„Tăul fără fund" de la Băgău — probe colectate în august 1991

12. mase gelatinoase colectate în zona periferică;
13. mase gelatinoase colectate în zona centrală.

Materialul biologic prelevat a fost fixat în teren cu formaldehidă 4%, identificarea speciilor de alge efectuându-se în laborator.

Pentru stabilirea gradului de similaritate floristică am utilizat indicele lui Sörensen: $P = \frac{2c}{a+b} \cdot 100$, în care P = similaritate exprimată în procente, c = nr. specii comune în cele două comunități (A și B) care se compară, a = nr. specii în comunitatea A, și b = nr. specii în comunitatea B. Prelucrarea datelor s-a efectuat pe baza unui program de calcul, în limbaj BASIC, conceput pentru un microcalculator personal TIM-S.

Rezultate și discuții. În urma identificării algelor din cele două mlaștini am constatat că „Tăul fără fund" prezintă o floră algală mult mai săracă în specii (68) și mai uniformă decât cea de la Pesteana, unde numărul speciilor se ridică la 152 (Tabel 1). Deoarece flora algală a mlaștinii de la Pesteana a mai fost cercetată, așa cum am arătat (prima oară în 1880) și datorită faptului că în lucrarea de față vom folosi și eșantioanele colectate în 1968, pe lângă cele din 1991, în timp ce mlaștina de la Băgău este la prima cercetare din acest punct de vedere, vom încerca o caracterizare a florei algale separat, pe fiecare mlaștină, și numai în final vom realiza o comparare a comunităților algale per global, pe baza indicelui de similaritate.

Distribuția procentuală pe grupe sistematice a florei algale din mlaștina de la Băgău (68 specii), este următoarea:

Cyanophyta — 5%
Chrysophyta — 7%
Xanthophyta — 1%
Bacillariophyta — 35%
Chlorophyta-Chlorococcales — 16%
Chlorophyta-Desmidiales — 20%
Euglenophyta — 13%
Dinophyta — 3%

Remarcăm procentul ridicat de diatomee și desmidiacee, dar și de clorofite. Aproximativ jumătate din algele identificate sunt specii larg răspândite în mlaștinile de turbă oligo- și mezotrofe din țară [2—5]. Cu toate acestea, numărul speciilor sfagnobionte și sfagnofile este relativ mic. Acestea sunt: *Chroococcus minutus, Chrysastrella paradoxa, Actinotaenium cucurbita, Myxochloris sphagnicola, Oocystis solitaria, Eunotia exigua, E. fallax, E. pectinalis, E. sudetica, E. tenella, E. veneris, Pinnularia microstauron, P. termitina, P. viridis, Gloeodinium montanum.* Menționăm numărul redus sau absența totală a unor desmidiacee și diatomee sfagnicole din genurile: *Staurastrum, Euastrum, Closterium,*

Tabel 1

Structura comunităţilor algale din mlaştinile de la Poşteana şi „Tăul fără fund" de la Băgău

Taxoni	Habitate												
	1	2	3	4	5	6	7	8	9	10	11	12	13
CYANOPHYTA													
Anabaena augstumnalis	+	+	+		+		+	+		+		+	
Anabaena solitaria	+	+	+		+		+						
Anabaena verrucosa				+		+							
Aphanothece microscopica								+	+				
Chroococcus haematoides								+					
Chroococcus minutus	+		+	+		+		+	+	+	+	+	+
Chroococcus montanus									+				
Chroococcus turgidus			+	+	+	+	+		+				
Coelosphaerium kuetzingianum								+				+	
Cylindrospermum minutissimum									+				
Cylindrospermum muscicola						+							
Merismopedia glauca			+										
Merismopedia minima						+	+						
Nostoc microscopicum							+						
Oscillatoria tenuis								+			+		
Phormidium tenue								+					
Synechococcus aeruginosus				+			+		+				
CHRYSOPHYTA													
Chrysastrella paradoxa						+			+	+	+	+	+
Chrysococcus rufescens	+							+	+	+	+	+	+
Dinobryon divergens													+
Dinobryon sertularia										+	+	+	
Epipyxis utriculus										+	+	+	+
Lepochromulina calix											+		
Monosiga varians										+	+		
XANTHOPHYTA													
Heterodendron squarosum		+			+	+							
Myxochloris sphagnicola												+	
Ohiocytium cochleare	+						+						
BACILLARIOPHYTA													
Achnanthes linearis								+					
Achnanthes marginulata											+	+	
Amphora ovalis							+						
Amphora perpusilla							+						
Caloneis amphisbaena							+						
Caloneis bacillum							+						
Caloneis silicula											+		
Cocconeis placentula							+						
Cymatopleura solea							+						
Cymbella aspera							+						
Cymbella gracilis	+	+	+	+	+								
Cymbella perpusilla					+								
Cymbella prostrata							+						
Cymbella tumida							+	+					
Cymbella ventricosa											+		
Diatoma hiemale							+						
Epithemia ocellata											+		
Epithemia zebra							+						

Tabel 1 (continuare)

Taxoni	1	2	3	4	5	6	7	8	9	10	11	12	13
Eunotia curvata	+	+	+	+	+			+	+	+	+	+	+
Eunotia exigua		+	+	+	+	+	+	+	+	+	+	+	+
Eunotia fallax et v. gracillima					+				+	+	+	+	+
Eunotia pectinalis		+						+			+	+	
Eunotia sudetica										+	+		+
Eunotia tenella	+	+	+	+	+	+	+	+	+	+	+	+	+
Eunotia veneris								+					
Fragilaria pinnata							+						
Frustulia saxonica	+	+	+	+	+	+		+	+				
Frustulia vulgaris								+	+				
Gomphonema acuminatum								+					
Gomphonema angustatum											+		
Gomphonema constrictum							+						
Gomphonema olivaceum							+						
Gomphonema parvulum	+			+			+	+			+		
Gyrosigma scalproides							+						
Gyrosigma spencerii							+				+		
Hantzschia amphioxys							+	+					+
Melosira granulata							+						
Melosira italica							+						
Navicula cryptocephala							+						
Navicula cuspidata							+						
Navicula forcipata							+						
Navicula gracilis							+	+					
Navicula hungarica							+						
Navicula lanceolata							+						
Navicula pupula							+				+		
Navicula radiosa							+						
Navicula reinhardtii							+						
Navicula rhyncocephala							+						
Navicula roteana							+						
Navicula subtilissima		+	+	+		+	+		+				
Navicula viridula							+						
Neidium bisulcatum											+		
Nitzschia amphibia							+						
Nitzschia denticula							+						
Nitzschia fonticola								+					
Nitzschia heufleriana							+						
Nitzschia microcephala							+						
Nitzschia palea		+	+	+	+		+	+			+		
Nitzschia sigmoides							+						
Pinnularia abaujensis								+			+		
Pinnularia biceps f. petersenii								+					
Pinnularia borealis					+			+					
Pinnularia braunii				+				+					
Pinnularia brebissonii	+	+						+					
Pinnularia esox								+					
Pinnularia hilseana								+			+		
Pinnularia interrupta								+					
Pinnularia major		+	+					+					+
Pinnularia mesolepta	+	+						+		+	+		
Pinnularia microstauron		+			+			+			+		
Pinnularia nobilis	+	+	+							+	+	+	+
Pinnularia rupestris		+											
Pinnularia ruttnerii	+	+	+										

Tabel 1 (continuare)

Taxoni	1	2	3	4	5	6	7	8	9	10	11	12	13
Pinnularia subcapitata	+												
Pinnularia sudetica	+	+	+		+			+			+	+	+
Pinnularia termitina	+	+	+	+	+	+	+	+	+	+	+	+	+
Pinnularia viridis et v. minor	+	+	+		+					+	+	+	+
Rhoicosphaenia curvata							+						
Rhophalodia gibba							+						
Tabellaria fenestrata							+						
Tabellaria flocculosa		+							+				
Stauroneis anceps											+	+	+
Stenopterobia intermedia	+	+	+										
Stephanodiscus astraea							+						
Stephanodiscus dubius							+						
Stephanodiscus hantzschii							+						
Surirella robusta							+						
Synedra ulna							+						
CHLOROPHYTA-CHLOROPHYCEAE													
Asterococcus superbus		+	+	+	+				+		+	+	
Botryoccocus braunii			+	+	+	+	+						
Chlamydomonas depressa											+		
Chlamydomonas tapeta										+	+	+	+
Desmatractum bipyramidatum									+				
Elakatothrix gelatinosa									+				
Enallax alpina				+									
Eremosphaera viridis			+		+	+				+	+		
Eudorina elegans	+	+	+		+								
Golenkinia radiata							+						
Hormidium flaccidum												+	+
Microspora pachyderma			+		+		+		+	+	+	+	
Microthamnion strictissimum								+	+				
Monoraphidium irregulare										+			
Oocystis solitaria				+		+	+		+		+		
Pandorina morum			+		+								
Sphaerellopsis fluviatilis											+		
Tetraedron incus											+		
CHLOROPHYTA-DESMIDIALES													
Actinotaenium cucurbita	+	+	+	+		+	+		+	+	+	+	+
Arthrodesmus incus				+		+	+		+		+		
Bambusina brebissonii			+										
Closterium dianae	+	+			+							+	
Closterium lineatus	+	+	+									+	
Closterium lunula	+	+	+	+									
Closterium striolatum	+	+	+	+					+			+	
Cosmarium botrys	+												
Cosmarium connatum									+				
Cylindrocystis brebissonii						+			+	+	+	+	+
Cylincrocystis crassa		+						+		+			
Desmidium swartzii		—	+	+	+	+							
Euastrum ansatum	+	+	+		+	+			+				
Euastrum binale	+			+		+	+		+				
Euastrum oblongum		+	+								+	+	+
Gonatozygon kinahanii	+												
Hyalotheca dissiliens		—	+		+								
Micrasterias rotata	+	+	+		+								

Tabel 1 (continuare)

Taxoni	1	2	3	4	5	6	7	8	9	10	11	12	13
Mougeotia parvula		+	+	+	+	+	+		+	+	+	+	+
Netrium digitus	+	+	+	+	+	+	+		+	+			
Netrium interrupta		+	+										
Penium phymatosporum						+							
Pleurotaenium minutum		+											
Pleurotaenium trabecula	+	+	+		+			+					
Straurastrum margaritaceum				+		+			+				
Straurastrum micron											+		
Staurastrum muricatum				+		+							
Staurastrum simonyi									+				
Staurastrum spinosum				+									
Teilingia granulata	+					+			+				
EUGLENOPHYTA													
Calycimonas physaloides								+	+				
Colacium sideropus								+					
Distigma protaeus								+					
Eugle a acus										+			
Euglena deses										+			
Euglena mutabilis	+		+	+		+			+	+	+	+	+
Menoidium pellucidum										+	+		
Petalomonas polytaphrena							+		+				
Phacus orbicularis								+		+	+		
Trachelomonas hyspida							+						
Trachelomonas verrucosa										+	+		
Trachelomonas volvocina				+				+	+	+	+	+	+
Trachelomonas volvocinopsis	+			+		+	+	+	+	+	+	+	
DINOPHYTA													
Gloeodinium montanum						+	+	+	+	+		+	+
Peridinium umbonatum	+		+	+		+	+		+				

Cosmarium, Frustulia, Navicula, Pinnularia, care vegetează în mod obișnuit în astfel de habitate [2—5]. În același timp, am observat frecvența mare a elementelor euritope sau chiar eutrofe, cenoxene, precum specii de: *Chlamydomonas, Trachelomonas, Monoraphidium, Sphaerellopsis, Tetraëdron, Hormidium* și *Euglena.*

Sub aspectul compoziției cantitative, comunitățile algale sunt dominate, în marea lor majoritate, de *Cylindrocystis brébissonii,* speciile subdominante fiind *Pinnularia termitina* și *Chlamydomonas tapeta.* Un număr mai redus de probe au fost dominate de *Chlamydomonas tapeta* cu *Cylindrocystis brébissonii* și *Pinnularia termitina* ca subdominante.

Prin numărul mic de specii de alge identificate și prin marea uniformitate constatată în cadrul comunităților algale, mlaștina de la Băgău constituie o situație particulară comparativ cu alte mlaștini similare din țară. Acest lucru este valabil chiar dacă o comparăm cu mlaștini situate aproximativ la aceeași altitudine, bunăoară cu cele de la Sălicea (840 m) [6, 9] sau din Mestecănișul de la Reci (220 m) [8], unde numărul algelor identificate (peste 200) și diversitatea floristică sunt mult mai mari.

Pentru stabilirea structurii comunităţilor algale din mlaştina de la Peşteana, ne-am folosit atât de materialul biologic colectat în vara anului 1991 (coloanele 8 şi 9 din Tabelul 1) cât şi de cele colectate în 1968 (coloanele 1—7 din Tabelul 1). În final, am identificat un număr de 152 taxoni per total, faţă de numai 32 taxoni, cât a citat Schaarschmidt în 1880 [14] (Tabel 2). În acelaşi timp, aproximativ 50% din speciile identificate de Schaarschmidt [14] sunt clorofite, toate algele în ansamblul lor fiind elemente care vegetează în habitate tipic eutrofe, comunitatea respectivă fiind caracteristică pentru bazinele acvatice lacustre.

Tabel 2

Algele identificate de Schaarschmidt (*1880*) [*14*] în „Tăul de la Paşteana"

CYANOPHYTA

Glocotrichia natans
Gomphosphaeria aponina
Merismopedia glauca
Rivularia radians
Tolypotrix aegarophila

XANTHOPHYTA

Ophiocytium cochleare

BACILLARIOPHYTA

Cymbella lanceolata
Hantzschia amphioxys
Pinnularia viridis
Stauroneis phoenicenteron
Synedra acus
Synedra ulna
Synedra radians

CHLOROPHYTA-CHLOROPHYCEAE

Ankistrodesmus aciculare
Ankistrodesmus minutum
Chaetophora pisiformis
Coelastrum sphaericum
Dictyosphaerium reniforme
Gongrosira ericetorum
Hydrodictyon utriculatum
Pandorina morum
Pediastrum boryanum
Pediastrum duplex
Polyedriopsis spinulosa
Pseudostaurastrum enorme
Scenedesmus obtusus
Tetraedron minimum
Ulothrix zonata

CHLOROPHYTA-DESMIDIALES

Mougeotia genuflexa
Spirogyra decinina
Spirogyra gracilis
Zygnema cruciatum

Paralel cu procesul de sfagnetizare a lacului, a avut loc şi schimbarea corespunzătoare a florei algale, astfel că algoflora pe care o prezentăm în Tabelul 1 are un pronunţat caracter mezo-oligotrof. Un singur eşantion se abate de la această situaţie, respectiv cel corespunzător coloanei 7 din Tabelul 1, care provine din ochiurile centrale ale mlaştinii (material colectat în 1968), ultimele rămăşiţe ale lacului de odinioară. Diferenţele dintre această probă şi restul comunităţii de la Peşteana se pot observa şi din distribuţia procentuală, pe grupe sistematice, a celor 72 taxoni identificaţi în acest eşantion, faţă de cei 112 din restul probelor:

Proba 7		Peșteana
Cyanophyta	5%	13%
Chrysophyta	1%	2%
Xanthophyta	—	2%
Bacillariophyta	66%	38%
Chlorophyta		
— Chlorococcales	12%	9%
— Desmidiales	8%	27%
Dinophyta	3%	2%
Euglenophyta	5%	7%

Procentul ridicat de diatomee euritope sau chiar eutrofe (*Amphora ovalis, A. perpusilla, Caloneis amphisbaena, C. bacillum, C. silicula, Cocconeis placentula, Cymatopleura solea, Cymbella aspera, C. tumida, C. ventricosa, Epithemia zebra, Gomphonema constrictum, G. olivaceum, G. parvulum, Gyrosigma scalproides, G. spencerii,* specii de *Navicula, Nitzschia, Stephanodiscus* etc.) și cel scăzut de desmidiacee diferențiază net comunitățile algale tipic eutrofe din proba 7 de cele mezo-oligotrofe din mlaștina de la Peșteana. În acest sens, remarcăm numărul mare de elemente sfagnofile și sfagnobionte identificate: *Chroococcus turgidus, Synechococcus aeruginosus, Chrysastrella paradoxa, Cymbella gracilis, C. perpusilla, Frustulia saxonica, Eunotia curvata, E. exigua, E. tenella, Navicula subtilissima, Pinnularia interrupta, P. esox, P. microstauron, P. termitina, P. subcapitata, P. sudetica, P. viridis, Tabellaria flocculosa, Stenopterobia intermedia, Asterococcus superbus, Botryococcus braunii, Eremosphaera viridis, Actinotaenium cucurbita, Bambusina brébissonii, Cylindrocystis crassa, Pleurotaenium minutum, Staurastrum margaritaceum, S. simonyi, S. spinosum, Oocystis solitaria, Calycimonas physaloides, Petalomonas polytaphraena, Gloeodinium montanum.* Speciile menționate au o largă răspândire în mlaștinile oligo- și mezotrofe din țară [2—5, 7]. Menționăm însă și faptul că o serie de desmidiacee de talie mare, aparținând genurilor *Micrasterias, Closterium, Cosmarium, Euastrum, Pleurotaenium, Penium, Tetmemorus*, sunt reprezentate slab sau lipsesc complet din flora mlaștinii de la Peșteana, mai ales din materialul colectat în 1991 (Tabel 1), alge care sunt prezente în număr mare în alte habitate similare [5, 6]. Cu toate acestea, sfagnetul cercetat este comparabil, din acest punct de vedere, cu alte mlaștini de turbă din țară, mai ales cu cele situate la aceeași altitudine, respectiv Sălicea [6, 9] și Mestecănișul de la Reci [8]. Numărul relativ mare de elemente euritope, unele cenoxene, identificate *(Anabaena verrucosa, Phormidium tenue, Diatoma hiemale, Elakatothrix gelatinosa* etc.) se datorește puternicului stres antropic exercitat asupra mlaștinii, prin pășunatul intensiv care se practică în zonă, precum și configurației terenului.

Mlaștina de la Peșteana este, în același timp, mult mai mozaicată, și prezintă o algofloră mai bogată și mai diversificată decât cea de la Băgău.

Speciile dominante în cadrul comunităţilor cercetate sunt cele sfagnofile sau sfagnobionte. Astfel, *Actinotaenium cucurbita* apare cu frecvenţă mare în unele probe, *Euastrum binale* şi *Actinotaenium cucurbita* în altele, iar *Pinnularia termitina* şi *Frustulia saxonica* domină net în alte eşantioane.

Urmărind evoluţia şi modificarea structurii comunităţilor algale din mlaştina de la Peşteana începând din 1880 [14] până în 1991, pe baza datelor prezentate mai sus, putem afirma cu certitudine că există o tendinţă clară de oligotrofizare a acesteia.

Analizând dendrograma din Fig. 1, care prezintă gradul de similaritate floristică dintre comunităţile algale din sfagnetele de la Peşteana

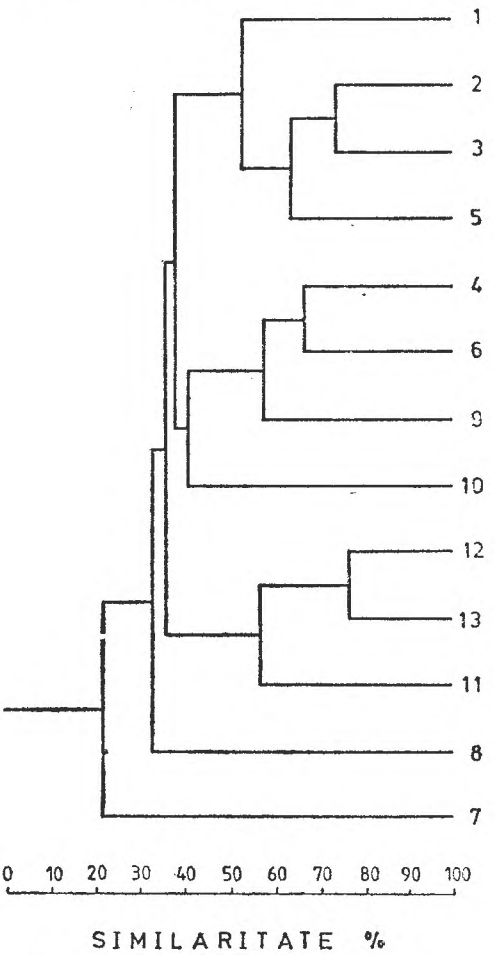

Fig. 1. *Dendrogramă prezentând gradul de similaritate floristică între comunităţile algale din sfagnetele de la Peşteana şi „Tăul fără fund" de la Băgău.*

și „Tăul fără fund" de la Băgău, constatăm conturarea clară a trei agregate (grupuri de probe) la un nivel de similaritate de peste 50%. O primă grupare (1, 2, 3, 5) este cea care reunește probele provenite, în general, din zona de lagg a mlaștinii de la Pește ana, din materialul colectat în 1968. În cel de-al doilea agregat (4, 6, 9), sunt cuprinse eșantioane ce reprezintă comunități algale similare de sfagnoplancton și mase gelatinoase de alge din mlaștină, dar colectată atât în 1968 cât și în 1991. De acest din urmă agregat se leagă proba 10, provenită de la Băgău, la un nivel de similaritate de 40%. Celelalte comunități algale, provenite din „Tăul fără fund" (11, 12, 13), se grupează în cel de-al treilea agregat. Menționăm că primele două agregate, care grupează comunitățile algale de la Pește ana (exceptând proba 10), se leagă între ele la 38% similaritate floristică, iar cel de-al treilea, care reunește comunitățile de la Băgău, se atașează de primele două la un nivel de 35% similaritate. Algele identificate din ceea ce a mai rămas din lagg-ul de altădată (proba 8) prezintă un indice de similaritate floristică cu ansamblul comunităților de mai sus de numai 32%. Complet diferită este comunitatea corespunzătoare eșantionului 7, care din motivele prezentate mai sus, realizează doar 22% similaritate floristică în raport cu celelalte comunități algale cercetate.

Concluzii. 1. Comunitățile algale care se dezvoltă în „Tăul fără fund" se caracterizează printr-un număr redus de specii și o mare uniformitate floristică, ceea ce conferă mlaștinii o poziție particulară față de alte habitate similare din țară.

2. Pe baza datelor prezentate se poate afirma că mlaștina de la Pește ana a prezentat în intervalul 1880—1991 o evoluție clară de la un ecosistem acvatic lacustru spre o mlaștină mezotrofă cu câteva enclave oligotrofe.

3. Indicele de similaritate floristică utilizat a permis evidențierea diferențelor existente între cele două mlaștini cercetate, precum și între florele algale existente în diferitele momente ale evoluției aceleași mlaștini (Pește ana 1968 și 1991).

4. Ambele mlaștini sunt supuse unui puternic stres antropic (datorat pășunatului intensiv la Pește ana sau/și adăpatului vitelor la Băgău), ceea ce se reflectă și în structura florei algale, în care sunt prezente o serie de elemente cenoxene.

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STUDIA UNIV. BABEŞ-BOLYAI, BIOLOGIA, XXXIX, 1, 1994

OBSERVAŢII FENOLOGICE ASUPRA UNEI PAJIŞTI MEZOFILE DIN BAZINUL SUPERIOR AL VĂII HUZII (MUNTELE SĂCEL, JUD. CLUJ)

IOAN WAGNER*

SUMMARY. — Phenological Observations on a Mesophilous Meadow from the Superior Basin of the Huzii Valley (Săcel Mountain, Cluj District). The paper presents the results of a phenological research, accomplished in a mesophilous meadow (ass. *Anthyllido-Festucetum rubrae* Soó 1971) from the superior basin of the Huzii Valley. The experimental area is situated on a southern aspect (25—30°), at 800 m a.s.l. Data are recorded between the beginning of April and the end of October, 1992. The phenophases, graphically represented, are influenced especially by temperature.

În decursul anului 1992 am efectuat observaţii fenologice asupra unei pajişti aparţinătoare asociaţiei *Anthyllido-Festucetum rubrae* Soó 1971, situată în bazinul superior al văii Huzii (Muntele Săcel, jud. Cluj), pe o expoziţie sudică, cu o înclinare a pantei de 25—30 de grade şi la o altitudine de 800 m.s.m. Metoda de cercetare utilizată este cea elaborată de D i e r s c h k e [2—6]. Observaţiile au fost efectuate pe teren din prima decadă a lunii aprilie până la sfârşitul lunii octombrie, la intervale de aproximativ două săptămâni.

Speciile edificatoare ale acestei pajişti sunt *Festuca rubra* şi *Agrostis capillaris*, alături de acestea acoperiri mari mai realizează şi *Anthyllis vulneraria, Anthoxanthum odoratum, Luzula campestris, Trifolium pratense* şi *Trifolium aureum.*

Pe baza speciilor de recunoaştere şi edificatoare, cu o fizionomie aparte, în pajiştea cercetată au fost puse în evidenţă 8 fenofaze, care sunt reprezentate grafic (Fig. 1).

Prima fenofază vernală debutează în ultima decadă a lunii aprilie, când temperaturile medii au trecut de +5°C şi pe panta respectivă exista o insolaţie destul de puternică. Speciile care înfloresc în această perioadă sunt *Primula officinalis, Ajuga reptans* şi *Carex pallescens.*

A doua fenofază, de asemenea vernală, debutează la începutul lunii mai, o dată cu înflorirea în masă a speciilor *Anthoxanthum odoratum, Luzula campestris, Fragaria viridis* şi *Cruciata laevipes.*

A treia fenofază este preestivală şi se declanşează la sfârşitul lunii mai. Se caracterizează prin începutul înfloririi speciilor *Anthyllis vulneraria, Trifolium aureum* şi *Genista sagittalis,* care vor domina fizionomic prin inflorescenţele lor galbene. De asemenea, mai înfloresc şi *Plantago lanceolata, P. media, Trifolium pratense* şi *Thymus pulegioides.*

A patra fenofază marchează începutul perioadei estivale şi se înregistrează în mijlocul lunii iunie, când temperaturile au marcat o creştere

* *Institutul de Cercetări Biologice, 3400 Cluj-Napoca, România*

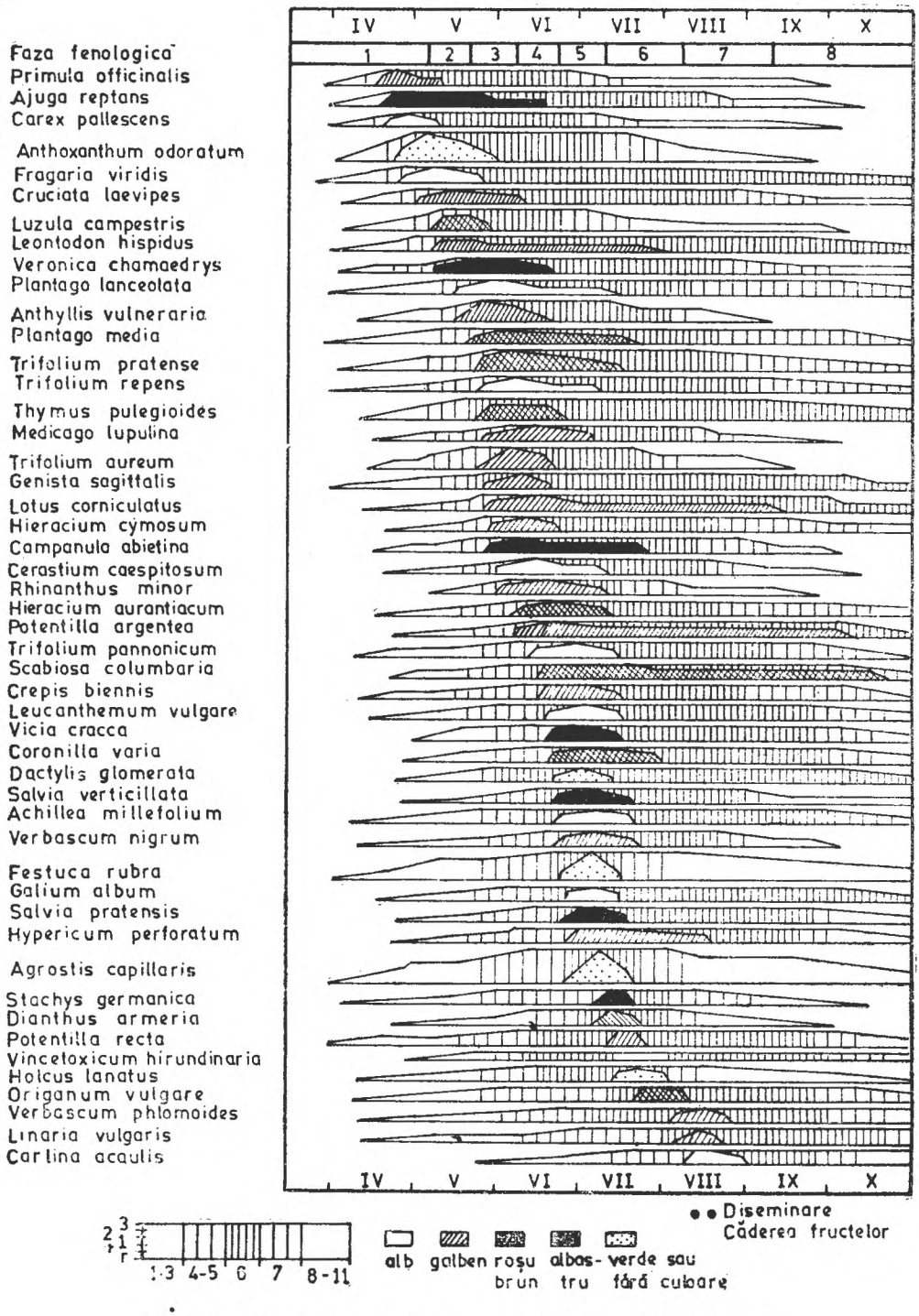

Fig. 1. *Diagrama simfenologică a principalelor specii din fâșiște.*

peste +10°C a mediei. Speciile *Anthyllis vulneraria* și *Trifolium aureum* ajung la dezvoltarea maximă, conferind pajiștii un aspect în care predomină culoarea galbenă. La aceasta contribuie și speciile *Medicago lupulina, Lotus corniculatus, Hieracium cymosum, Rhynanthus minor* și *Crepis biennis.*

Fenofaza a cincea este tot estivală și are loc de la sfârșitul lunii iunie până la începutul lunii iulie. Specii caracteristice care înfloresc în această perioadă sunt *Leucanthemum vulgare, Dactylis glomerata* și *Salvia verticillata.* Este începutul înfloririi speciilor edificatoare *Festuca rubra* și *Agrostis capillaris.*

A șasea fenofază este ultima fenofază estivală, în a doua și a treia decadă a lunii iulie și prima decadă a lunii august. În această perioadă se ating cele mai ridicate temperaturi medii din an. Speciile edificatoare *Festuca rubra* și *Agrostis capillaris* ajung la maximul înfloririi și al biomasei. Speciile care înfloresc sunt *Stachys germanica, Potentilla recta, Holcus lanatus* și *Oryganum vulgare.* La sfârșitul fenofazei se remarcă începutul declinului vegetativ al speciilor edificatoare din pajiște.

A șaptea fenofază, începe la sfârșitul lunii august și se continuă până în prima decadă a lunii septembrie. Temperaturile medii ridicate și precipitațiile reduse, la care a contribuit și insolația puternică din acest staționar sudic au dus la rapida îngălbenire a speciilor de graminee edificatoare. La sfârșitul fenofazei, pajiștea devine uscată și are un aspect gălbui. Printre puținele specii ce înfloresc în această perioadă se numără *Verbascum phlomoides, Linaria vulgaris* și *Carlina acaulis.*

Ultima fenofază este cea autumnală. Ea a început din mijlocul lunii septembrie, când temperaturile medii au înregistrat o bruscă scădere sub +10°C. Majoritatea speciilor de plante din pajiște și-au încheiat vegetația, fiind complet uscate. Se mai remarcă flori doar la specii semperflorente, la care înflorirea se eșalonează aproape pe întrega lor perioadă de vegetație, prelungindu-se până târziu în toamnă, precum *Scabiosa columbaria* și *Potentilla argentea.*

Din cercetările efectuate se desprind câteva remarci fenologice. Se observă că în pajiște perioada de vegetație este mai scurtă decât într-o pădure [1], din aceeași regiune. Intrarea în vegetație a speciilor din pajiște are loc la sfârșitul lunii aprilie în acest staționar cu expoziție sudică, chiar mai târziu în pajiști cu expoziție nordică din regiunea cercetată [1], în timp ce în făgetele din jur primele plante înfloresc în martie [1]. De asemenea, încheierea vegetației are loc mai devreme la plantele din pajiște decât la cele din făget, în staționarul analizat plantele încep să se îngălbenească la sfârșitul lunii iulie. În făgete plantele își prelungesc vegetația până după căderea frunzelor arborilor, în luna octombrie [1].

Atât la pajiștea analizată cât și în general la pajiști, delimitarea fenofazelor este dificilă, înflorirea majorității speciilor eșalonându-se într-o perioadă scurtă de 2—3 luni, în mai-iunie-iulie, când umiditatea și temperatura sunt optime pentru speciile mezofile din pajiște. La

sfârşitul verii, în această staţiune cu o puternică insolaţie, condiţiile devin prea aride pentru majoritatea speciilor.

Faţă de pajiştile similare analizate din regiune, dar cu expoziţie nordică, în pajiştea prezentată, intrarea în vegetaţie s-a produs cu aproximativ două săptămâni mai devreme [1]. De asemenea, faza de îngălbenire şi uscare survine mai repede în această pajişte.

În pajiştea analizată se remarcă ponderea mare a speciilor xerofile şi xeromezofile, precum *Anthyllis vulneraria*, *Thymus pulegioides*, *Dianthus armeria*, *Linaria vulgaris*, *Carlina acaulis*, *Verbascum nigrum* şi *V. phlomoides*, absente în alte pajişti din regiune [1]. Unele specii mezofile, abundente în alte pajişti din regiune, lipsesc în schimb în pajiştea studiată.

Concluzii. 1. Fenofazele sunt determinate şi declanşate de temperatură. O variaţie a acesteia, atât datorită factorilor climatici cât şi microclimatici, duce la o grăbire sau o întârziere a fenofazelor.

2. Condiţiile microstaţionale mai aspre duc la o comprimare a fenofazelor în pajişte. Perioada de vegetaţie este mai scurtă, începând mai târziu şi terminându-se mai devreme în pajişte decât în pădure.

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STUDIA UNIV. BABEŞ-BOLYAI, BIOLOGIA, XXXIX, 1, 1994

THE SPERMATOGENETIC CYCLE IN TERRESTRIAL ISOPODS UNDER THE CLIMATIC CONDITIONS OF ROMANIA. I. THE ANNUAL EVOLUTION OF THE SPERMATOGENETIC PHASES IN THE TESTICULAR FOLLICLES OF *PORCELLIO SCABER* LATR. *(CRUSTACEA : ISOPODA)*

CONSTANTIN CRĂCIUN*

SUMMARY. — For the evaluation of the entire annual spermatogenetic cycle in a representative of terrestrial isopods *(Porcellio scaber)*, I performed histological studies (serial sections) on 548 gonads obtained from mature male individuals collected directly from nature every week, for one year. Six characteristic spermatogenetic phases were evidenced, which succeed along a spermatogenetic cycle, similarly to a functional wave. The multitude of data were processed statistically and monthly tables and graphs were realized (with weekly details), which comprise the evolution of the 6 spermatogenetic phases of a year, distinctly for each one of the 6 testicular follicles of the gonad pair. I established that, under the climatic conditions of Romania, in *Porcellio scaber* Latr. there is a single spermatogenetic cycle per year. The dynamic processes of this cycle occur in the warm months of spring, summer and autumn and are separated for the next cycle by a winter diapause. In this cycle, the evolution of the 6 spermatogenetic phases in the 3 follicles of a gonad shows a functional asynchronism of 2 or 3 phases among the follicles of the same gonad, but a complete synchronism between the homologous follicles of the two symmetrical gonads.

The male reproductive system of terrestrial isopods consists of a pair of gonads, symmetrically placed on both sides of the digestive tract (Fig. 1). In its turn, each gonad is made up of 3 testicular follicles which open separately and at different levels in the anterior region of vas deferens —a segment that functions are seminal reservoir. At the tip of each follicle, there are several cell rows which constitute the androgenic gland. The 3 follicles were designated as I, II, and III, according to the place where they are attached to the seminal vesicle and beginning with the follicle from the front. Also, the gonad from the left of the digestive tract was marked with L, and the one on the right with R [4].

In isopods, spermatogenesis occurs in the 6 testicular follicles, from where the mature spermatozoa pass and accumulate in the anterior segment of vas deferens. They move along the lower segments of this channel and are expelled through the penis during mating. There is a functional interdependence among all these segments of the reproductive system [4].

* *Babeş-Bolyai University, Department of Biology, 3400 Cluj-Napoca, Romania*

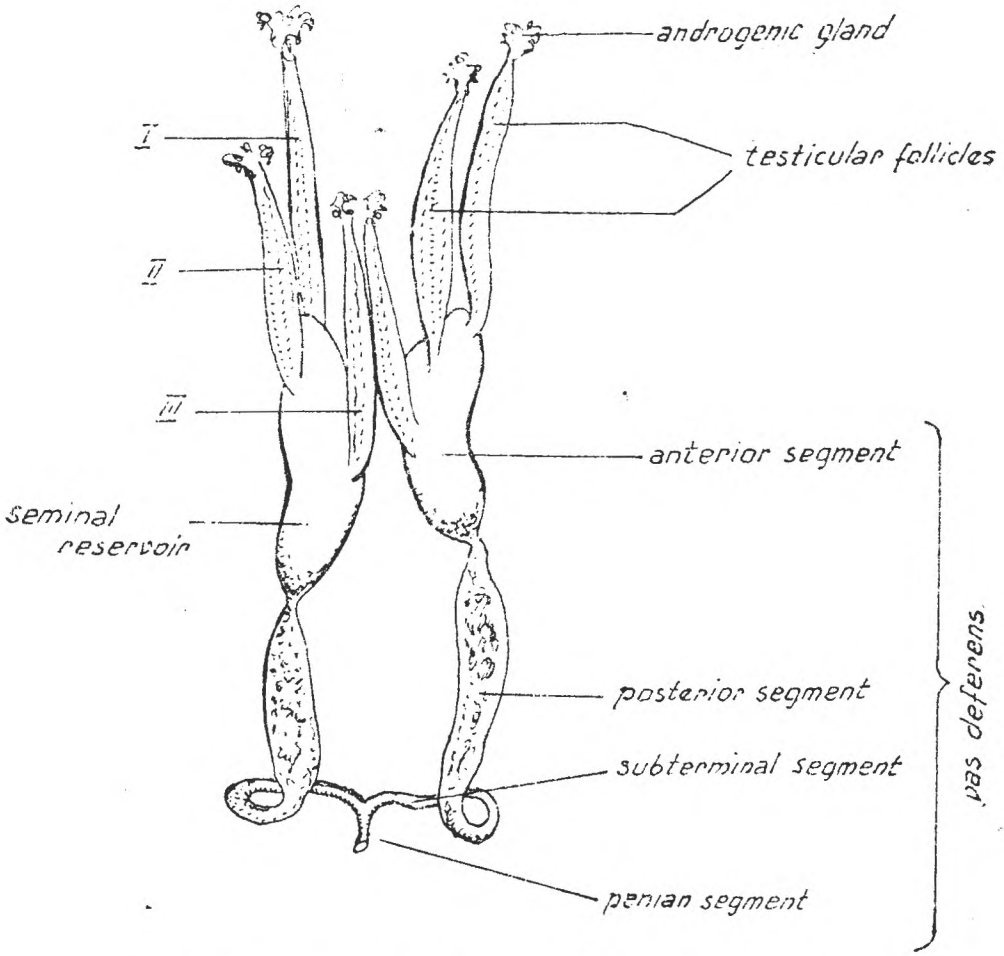


Fig. 1. *The male reproductive system in* Porcellio scaber *Latr.*

The existing studies on isopod spermatogenesis describe the spermatogenetic phases and the microscopic structure of their germinal elements [14, 30, 31, 33, 35, 44, 47], including the ultrastructure of the spermatozoon [2—5, 14, 16, 41—43]. Ample studies have also dealt with the structure and ultrastructure of vas deferens, mainly in *Porcellio scaber* [4, 7, 29, 32, 34, 36—38, 47], as well as with the androgenic gland [4, 6, 8, 9, 39].

These data on the structure and ultrastructure of the entire reproductive system of terrestrial isopods were supplemented with ecological information on their biology [40, 45, 46], and with similar data on other crustaceans [1, 25, 26, 28], which facilitated the establishment of multiple functional correlations about the physiology of isopod reproduc-

tion [4]. But, although these numerous studies clarified many aspects of the biology of terrestrial isopod reproduction, there are still many obscure aspects, such as: the length of a spermatogenetic cycle; the evolution of the spermatogenetic phases during a single spermatogenetic cycle and during a whole year in every of its various seasons; the functional synchronism or asynchronism of the two symmetrical gonads, as well as of the 6 testicular follicles; the evolution of spermatogenetic phases of animals kept under laboratory conditions during their normal hibernating rest.

All these problems have not been studied yet in their evolutive-cyclic complexity during a year, either in isopods or in other species of *Crustacea*. It is relevant in this case the study of F a i n-M a u r e l [14] on spermatogenesis in *Oniscus* and *Armadillidium* isopods, who admits the conclusions that spermatogenetic cycles succeed many times, but their number is impossible to be determined. A single report deals with the annual cycle of the spermatogenetic phases in *Armadillidium vulgare*, the study undertaken by R a d u and C r ă c i u n [35], but unfortunately, this research is short and irrelevant due to the small number of individuals studied.

Materials and methods. The gonads of 5—6 *Porcellio scaber* mature male individuals were excised every 6—7 days, for one year, 56 excisions in all. The gonads were prepared for histologic and cytologic observations, and serial sections of each gonad were examined under the light microscope. Additionally, male gonads were excised monthly and processed for electron microscopic studies.

As the study of the excised material progressed, I noticed that the most complete data on activity of testicular follicles were offered by the observations under the light microscope, because this microscope gave me the possibility to monitor the evolution of the entire gonad and to obtain numerous data which indicate the activity of a whole gonad at a given time. Conversely, the electron microscope required a lot of materials and time for an evolutionary study of the activity of the 6 testicular follicles of a single isopod, since it allowed only the examination of a very small part of the gonad, for which, nevertheless, it offered important details. That is why I focussed mainly on the observations under the light microscope for the study of the annual evolution of the activity of testicular follicles. Thus, I studied tens of thousands of serial sections through the 1644 testicular follicles of the 548 male gonads excised.

I examined separately the evolution of the spermatogenetic activity of the testicular follicles nos. I, II, and III of the left and right gonads. Alongside of the annual structural and functional evolution of the germinal elements, the degree of functional synchronism and asynchronism of the 6 testicular follicles for the two gonads of each of the *Porcellio scaber* individuals sacrificed was also studied.

Results and discussions. Testicular follicles are the structural and functional units in which spermatogenesis takes place, beginning with spermatogonia and ending with mature spermatozoa which group in clusters of 6—8 in spermatophores. In order to follow the dynamics of all the spermatogenetic processes in their chronologic evolution throughout a year, I defined, based on my previous studies [4], 6 phases which were characteristic of the whole spermatogenetic cycle. In chronologic order, these phases are (Fig. 2):

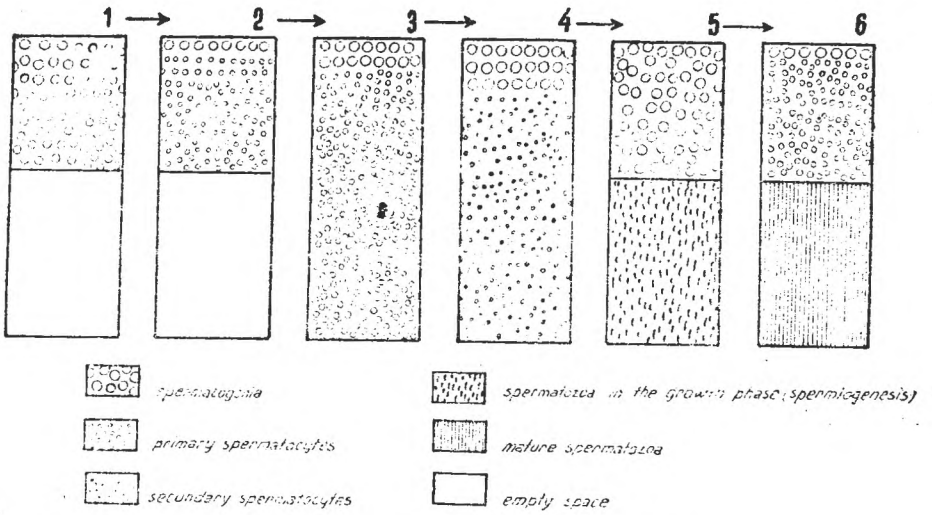


Fig. 2. *The spermatogenetic phases* (1 6).

— phase 1— spermatogonia are present in the upper half of the follicle; its lower half is empty;

— phase 2 — primary spermatocytes occupy the upper half of the follicle while the lower half is still empty;

— phase 3 — the whole follicle is full of primary spermatocytes;

— phase 4 — the whole follicle is filled with secondary spermatocytes or with spermatids; in the apical region there begins the proliferation of a new generation of spermatogonia;

— phase 5 — the apical half of the follicle is filled with spermatogoni; the dower half contains spermatozoa in different stages of development;

— phase 6 — in the apical half primary spermatocytes replaced spermatogonia, while in the lower half there are only mature spermatozoa grouped in spermatophores.

The sequence of these 6 spermatogenetic phases was established based on examinations of serial sections through each gonad with its 3 follicles. This sequence also presents intermediary stages that are halfway between the described phases of the spermatogenetic cycle. Thus, in winter, the follicles are usually in phase 1, with spermatogonia in the apical part and an empty space in the lower part. This phase, characteristic of the hibernal rest period, is caused by a degeneration of the germinal elements from the lower part of the follicles. Once the spring comes, the spermatogonia grouped in the upper parts of the follicles divide intensely and form the primary spermatocytes, which gradually fill the follicles completely (phases 2 and 3).

As the primary spermatocytes divide, the follicles become filled with secondary spermatocytes, which, in their turn, undergo a second

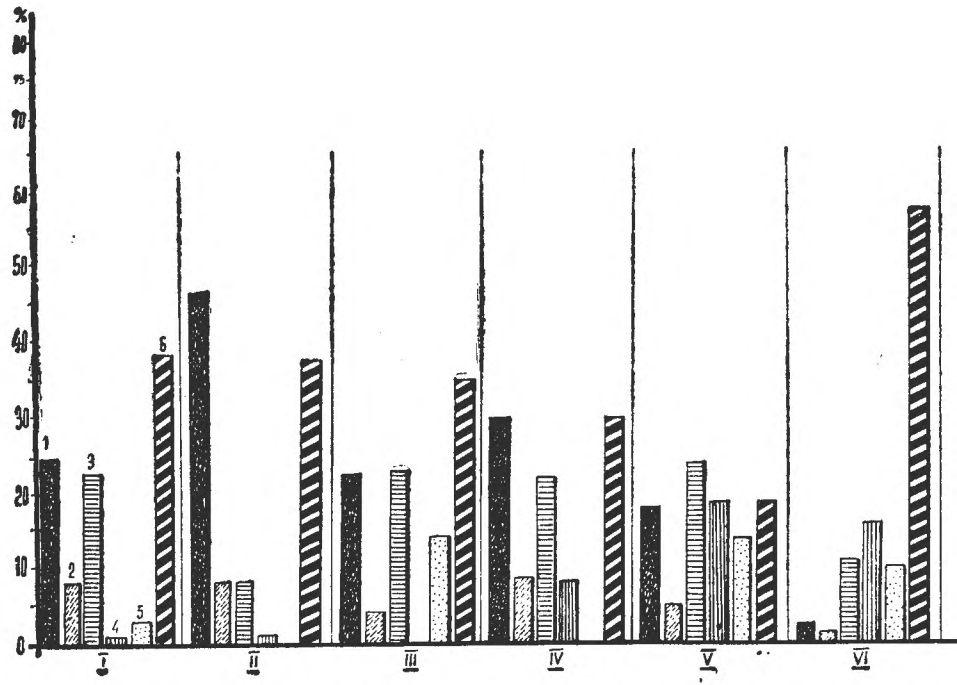
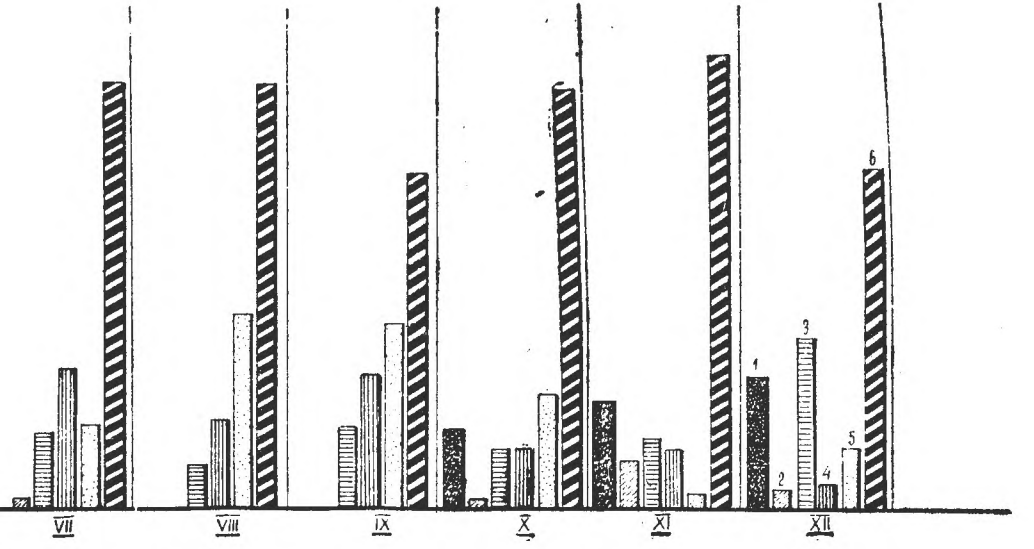


Fig. 3. *Monthly amplitude of the spermatogenetic phases* (1–



–6) *in* Porcellio scaber *Latr. Cummulative mean of the 3 follicles* (%).

maturation division and form spermatids (phase 4). Spermiogenesis begins with a change in the shape of the spermatids: they assume the form of a pear, then lengthen gradually (phase 5). The spermatogenetic cycle ends with the formation of mature spermatozoa and their clustering in spermatophores (phase 6). The spermatophores are then eliminated in the anterior segment of vas deferens, which acts as a seminal reservoir, while a new spermatogenetic cycle begins in each follicle.

Usually, the germinal elements of phases 2 and 3, formed in the lower part of the follicles in autumn, degenerate during winter, empting this part and leading to phase 1 follicles. In the follicle which begins in winter with mature spermatozoa, the majority of them are preserved and will constitute the first supply of spermatozoa required by mating in the following spring.

The 3 follicles of a gonad are rarely in the same phase. Most often, they are in 2 or 3 different phases. There are 3 possible situations:

— 1. the 3 follicles of a gonad are in the same spermatogenetic phase;

— 2. two follicles are in a same phase, while the third is in another phase;

— each follicle is in a different spermatogenetic phase.

Since I showed that the germinal elements pass through 6 phases in a spermatogenetic cycle, the problem is whether the 3 follicles of a gonad are in phases close or distant from one another, *i. e.* the degree of synchronism and asynchronism among the phases of the 3 follicles. If I consider the spermatogenetic phases in their chronologic order as deriving from one another, *i.e.*: 1—2—3—4—5—6—1—2—3— . . . etc., I will notice that, theoretically, there are 5 degrees of phase asynchronisms: first degree asynchronisms, which exist between two consecutive phases (1—2; 2—3; 3—4; 4—5; 5—6, as well as 6—1); second degree asynchronisms, when there is a difference of two phases (1—3; 2—4; 3—5; 4—6); third degree asynchronisms, when the difference consists of 3 phases (1—4; 2—5; 3—6); fourth degree asynchronisms, when there is a difference of 4 phases (1—5; 2—6); fifth degree asynchronisms, when there is a 5-phase difference (1—6). In fact, the fourth and fifth degrees are extremely rare, probably aberrant, as we shall see.

Another question that must be answered refers to the degree of synchronism or asynchronism between the homologous follicles of the two symmetrical gonads. This problem can be explained only by directly comparing the spermatogenetic phases from the homologous follicles belonging to the two symmetrical gonads, at a given moment.

In order to have as a complete image as possible of the spermatogenetic activity of the testicular follicles throughout a year, and to make significant functional correlations, all observations of all the microscopic preparations were grouped in monthly tables, where the following aspects were weekly analyzed and processed statistically:

1. the phase distribution of the germinal elements from the follicles;

2. the number of phases represented by the follicles of the same gonad;

3. the phase difference between the follicles of two gonads;
4. a comparison of phase synchronism and asynchronism degree among the follicles belonging to the same gonad;
5. the degree of phase synchronism and asynchronism between the homologous follicles of two symmetrical gonads.

The results obtained by studying these parameters are presented in 12 monthly tables, with weekly details on each sample (see Tables 1—12).

The data provided by weekly samplings of the isopods throughout the year, and presented in Tables 1—12, were processed statistically and I obtained a synoptic table (Table 13). This table allows us to view all the spermatogenetic activities occurring in a year. Also, in addition to the conclusions drawn for each month, the data from the synoptic table offers us the possibility to follow evolution of spermatogenesis throughout a year as well as the annual evolution of each phase in each follicle. This allows us to outline many functional aspects which occur in the testicular follicles during a year, and this knowledge facilitates our understanding of the functional correlations which are established in the whole male reproductive system.

The graphic representation of the data from this table indicate that the spermatogenetic phases are unequal in amplitude and evolution in time (Fig. 3). Of all the follicles analyzed in a year, 45% were in phase 6, while the remaining 55% contained germinal elements in the other 5 spermatogenetic phases (Fig. 4).

Looking to the annual phase distribution in Table 13, we notice that phase 6 is well represented in winter months, too, which is not the case with the other 5 phases. Since in April and May, the percentage of phase 6 is reduced to 17%, these data lead to the following conclusion: in April — May the isopods mate and fertilization occurs, involving — first — the spermatozoa preserved over winter in vas deferens and follicles. Therefore, in the basal area of the follicles, the germinal elements which underwent the fewest degenerations in winter are the spermatozoa from the spermatophores. In the apical part of the follicles, the germinal elements which are preserved best over winter are phase 1 elements, *i.e.* spermatogonia, which initiate a new spermatogenetic cycle in spring. I also noticed that the spermatozoa deposited in the anterior segment of vas deferens were well preserved over winter. This was possible because they were supplied with special secretory products called Eracosomes [7] elaborated by the glandular cells of vas deferens.

The graph of the monthly percentage data on the 6 spermatogenetic phases shows the curves of the evolution and amplitude of these phases for one year (Fig. 5). This graph makes it clear that the spermatogenetic phases marked 1, 2, 4 and 5 are not present in the follicles all the time. Phases 1 and 2 are missing in summer, phase 4 is absent in winter, and phase 5 is suspended in January, February, April and November. Phase 3 is represented throughout the year, with a moderate or low amplitude. Phase 6 is also present the whole year, always with a high amplitude, except in May.

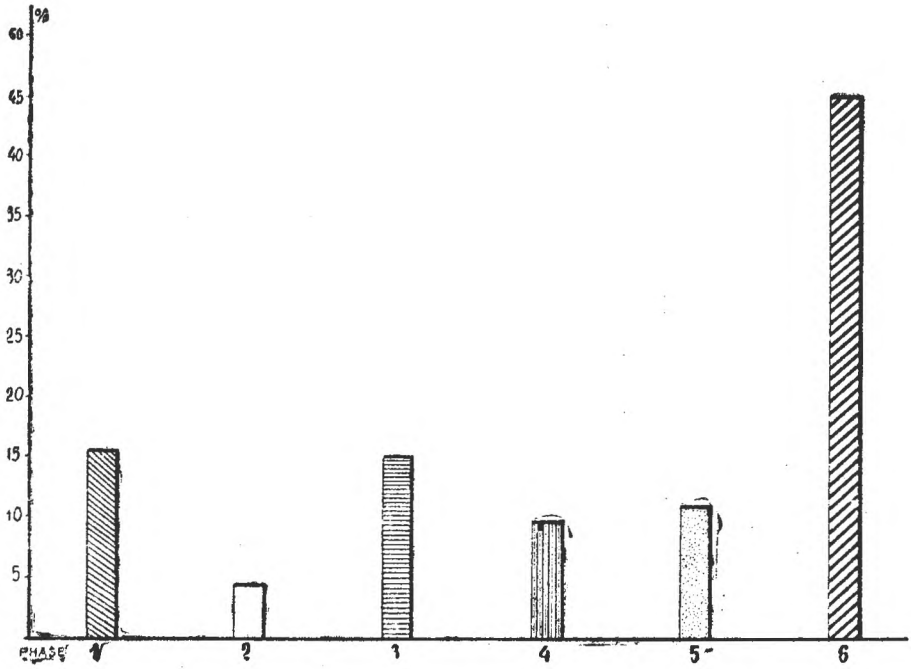


Fig. 4. *Amplitude of the spermatogenetic phases in* Porcellio scaber *Latr. throughout the year* (%).

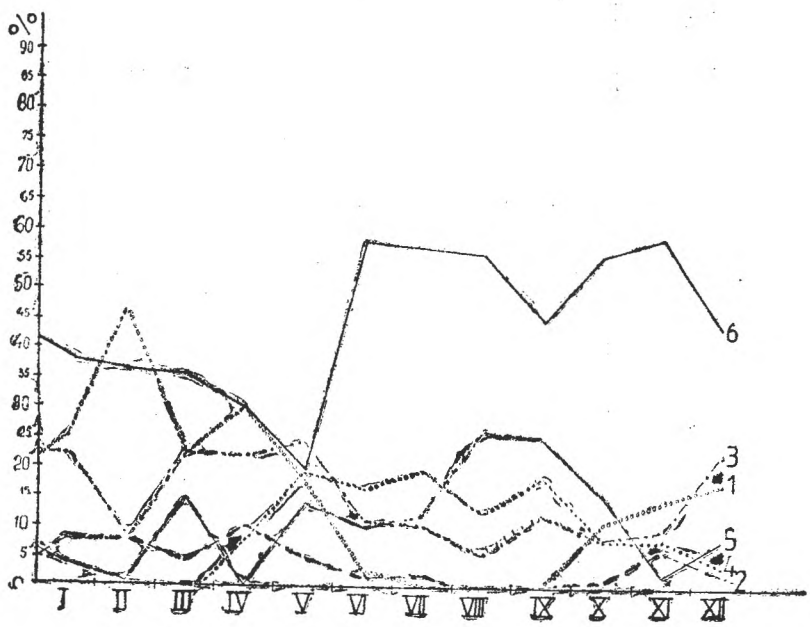


Fig. 5. *Evolution and amplitude of the spermatogenetic phases in* Porcellio scaber *Latr. throughout the year* (%).

Table 1

The structural and functional evolution of the testicular follicles in January

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
J.8	6	4/5	3	6	4	3	3	3	0—1/II	1—2	3	1—2	2	3	1	0	0—1	0
	6	3	1	6	3	1	3	3	0	3	1	2	3	1	2	0	0	0
	1	1	6	1	1	6	2	2	0	0	1	1	0	1	1	0	0	0
	2/3	6	6	2/3	6	6	2	2	0	2—3	2—3	0	2—3	2—3	0	0	0	0
J.17	1	2	2	6	2	2	2	2	1/I	1	1	0	2	2	0	1	0	0
	6	5	3	6	5/6	3	3	3	0—1/II	1	3	2	0—1	3	2—3	0	0—1	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	6	1	1	6	1	1/3	2	2	0—1/III	1	1	0	1	1	0	0	0	0—1
	3	6	1	3	6	1	3	3	0	3	2	1	3	2	1	0	0	0
J.23	4/5	2	6	3	6	6	3	2	1—2/I 2/II	2—3	1—2	2	3	3	0	1—2	2	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	2	6	1	2	6	1	3	3	0	2	1	1	2	1	1	0	0	0
J.30	6	4/5	6	6	1	6	3	2	2—3/II	1—2	0	1—2	1	0	1	0	2—3	0
	3	6	1	3	6	1	3	3	0	3	2	1	3	2	1	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	3	6	1/5	3	6	1/5	3	3	0	3	2	1	3	2	1	0	0	0

In January I analyzed 17 gonads with 34 testicles and 102 follicles (51 pairs).

Conclusions.

1. The follicles are in all the spermatogenetic phases, with the following distribution:

Phase									SPERMIOGENESIS					
Follicles	1	1/3		2/3	3	4	4/5	Total follicles	5	5/1	5/6	6	Total follicles	Total general
I	11	—	2	2	7	—	1	23	—	—	—	11	11	34
II	5	—	3	—	10	1	2	21	1	—	1	11	13	34
III	9	1	2	—	4	—	—	16	—	2	—	16	18	34
Total follicles	25	1	7	2	21	1	3	60	1	2	1	38	42	102
%	24.52	1	6.84	1.96	20.65	1	2.94	58.82	1	1.96	1	37.22	41.18	100

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phase; b) 10 testicles (30%) have follicles in two different phases; c) 24 testicles (70%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among follicles I, II and III:

	left	right
— synchronism	5 follicles = 9.80%	6 follicles = 11.76%
— asynchronism of 1/2 phase =	—	1 follicles = 1.96%
— asynchronism of 1 phase =	20 follicles = 39.22%	17 follicles = 33.33%
— asynchronism of 2 phases =	15 follicles = 29.41%	14 follicles = 27.45%
— asynchronism of 3 phases =	11 follicles = 21.57%	13 follicles = 25.50%
Total	51 follicles = 100%	51 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 44 pairs (86.28%) are synchronous; 7 pairs (13.72%) present the following asynchronism: 3 cases (5.88%) with 1/2 phase (2/II and 1/III); 1 case (1.96%) with 1 phase (1/I); 1 case with 1 and 1/2 phase (1/I); 1 case with 2 phases (1/II); 1 case with 2 and 1/2 phases (1/II). The frequency of modifications: 2/I, 4/II, 1/III.

Table 2

The structural and functional evolution of the testicular follicles in February

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
F.7	6	1	1	6	1	1	2	2	0	1	1	0	1	1	0	0	0	0
	1	6	1/3	1	6	1	2	2	1/III	1	0–1	1	1	0	1	0	0	1
	6	6/1	1	6	1/3	1	2	3	1/III	0–1	1	0–1	1	1	0–1	0	1	0
	1	1	6	1	3	6	2	3	2/II	0	1	1	2	1	3	0	2	0
	1	6	3	1	6	3	3	3	0	1	2	3	1	2	3	0	0	0
F.14	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0
	3	1	6	3	1	6	3	3	0	2	3	1	2	3	1	0	0	0
	6/1	1	1	6	1	3	2	3	0–1/I, 2/III	0–1	0–1	0	1	3	2	0–1	0	2
	2	1	1	2	1	1	2	2	0	1	1	0	1	1	0	0	0	0
	6	4/5	2	6	1	6/2	3	3	2–3/II, 2/II	1–2	2	2–3	1	1	1	0	2–3	2
F.20	6	3	1	6	3	1	3	3	0	3	1	2	3	1	2	0	0	0
	2	6	6	2	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	2	6	6	2	6	6	2	2	0	1	0	1	1	0	1	0	0	0
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0
F.26	3	6	1	1	1	6	3	2	2/I, 1/II, III	3	2	1	0	1	1	2	1	1
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0

In February I analyzed 19 gonads with 38 testicles and 114 follicles (57 pairs).

Conclusions.

1. The follicles lack the germinal elements of phases 4 and 5, while phase 3 elements are usually degenerating. The follicles has the following distribution:

Phase / Follicles	1	1/3	2	3	4/5	Total follicles	SPERMIOGENESIS 6	6/1	6/2	Total follicles	Total general
I	19	—	6	3	—	28	9	1	—	10	38
II	11	1	—	3	1	16	21	1	—	22	38
III	23	1	1	3	—	28	9	—	—	10	38
Total follicles	53	2	7	9	1	72	39	2	1	42	114
%	46.49	1.76	6.14	7.89	0.88	63.16	34.20	1.76	0.88	36.84	100

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 25 testicles (65.79%) have follicles in two different phases; c) 13 testicles (34.21%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

	left	right
— synchronism =	12 follicles = 21.05%	12 follicles = 21.05%
— asynchronism of 1/2 phase =	5 follicles = 8.71%	1 follicle = 1.75%
— asynchronism of 1 phase =	25 follicles = 43.86%	30 follicles = 52.63%
— asynchronism of 1—1/2 phase =	1 follicle = 1.75%	— — —
— asynchronism of 2 phases =	9 follicles = 15.80%	9 follicles = 15.80%
— asynchronism of 2—1/2 phases =	1 follicle = 1.75%	— — —
— asynchronism of 3 phases =	4 follicles = 7.02%	5 follicles = 8.77%
Total	57 follicles = 100%	57 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 47 pairs (82.46%) are synchronous; 10 pairs (17.54%) present the following asynchronism: 1 case (1.75%) with 1/2 phase (1/I); 4 cases (7.02%) with 1 phase (2/II, 2/III); 4 cases (7,02%) with 2 phases (1/I, 1/II, 2/III); 1 case (1.75%) with 2—1/2 phase (1/II). The frequency of modifications (asynchronisms): 2/I; 4/II; 4/III.

Table 3

The structural and functional evolution of the testicular follicles in March

Date	Phase of development of germinal element in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
M.6	3	1	1	3	1	1	2	2	0	2	2	0	2	2	0	0	0	0
	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	2	6	6	2	6	6	2	2	0	2	2	0	2	2	0	0	0	0
M.12	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	3	6	1	3	6	1	3	3	0	3	2	1	3	2	1	0	0	0
	6	6	1	6	6	1	2	2	0	0	0—1	1	0	1	1	0	0	0
M.21	4/5	6	5	4/5	6	5	3	3	0	1—2	0—1	1	1—2	0—1	1	0	0	0
	6	5	5	6	5	5	2	2	0	1	1	0	1	1	0	0	0	0
	5	3	6	5	3	6	3	3	0	2	1	3	2	1	3	0	0	0
M.26	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	3	1	6	3	1	6	3	3	0	2	3	1	2	3	1	0	0	0
	3	1	1	3	1	1	2	2	0	2	2	0	2	2	0	0	0	0
	5	2	6	5	2	6	3	3	0	3	1	2	3	1	2	0	0	0

In March I analyzed 16 gonads with 32 testicles and 96 follicles (48 pairs).

Conclusions.

1. The follicles present all the phases except phase 4 and have the following distribution:

Phase						SPERMIOGENESIS			
Follicles	1	2	3	4/5	Total follicles	5	6	Total follicles	Total general
I	8	2	12	4	26	2	4	6	32
II	6	2	10	—	18	2	12	14	32
III	8	—	—	—	8	6	18	24	32
Total follicles	22	4	22	4	52	10	34	44	96
%	22.92	4.17	22.92	4.17	54.17	10,42	35.41	45.83	100

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 12 testicles (37.50%) have follicles in two different phases; c) 20 testicles (62.50%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

	left	right
— synchronism =	6 follicles = 12.50%	6 follicles = 12.50%
— asynchronism of 1/2 phase =	1 follicle = 2.08%	1 follicle = 2.08%
— asynchronism of 1 phase =	14 follicles = 29.17%	14 follicles = 29.17%
— asynchronism of 1—1/2 phase =	1 follicle = 2.08%	1 follicle = 2.08%
— asynchronism of 2 phases =	15 follicles = 31.25%	15 follicles = 31.25%
— asynchronism of 3 phases =	11 follicles = 22.92%	11 follicles = 22.92%
Total	48 100%	48 100%

4. There is a perfect synchronism between the left and the right part of the gonad, all the homologous follicles (pairs) of the two testicles presenting the same phase.

Table 4

The structural and functional evolution of the testicular follicles in April

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
A.2[illegible]	3	6	1	3	6	1	3	3	0	3	2	1	3	2	1	0	0	0
	6	1	1	6	1	1	2	2	0	1	1	0	1	1	0	0	0	0
	2	2	2	5/6	3	2	1	3	2—3/I, 1/I	0	0	0	2—3	2—3	1	2—3	1	0
	6	5/6	1	6	5/6	1	3	3	0	0—1	1	1—2	0—1	1	1—2	0	0	0
	6	1/2	3	6	1/2	3	3	3	0	1—2	3	1—2	1—2	3	1—2	0	0	0
A.10	6	1	4	6	1	4	3	3	0	1	2	3	1	2	3	0	0	0
	6	1	3	6	1	3	3	3	0	1	3	2	1	3	2	0	0	0
	6	1	3	6	1	3	3	3	0	1	3	2	1	3	2	0	0	0
	6	3	2	6	3	2	3	3	0	3	2	1	3	2	1	0	0	0
	3/4	2	1	3/4	2	1	3	3	0	1—2	2—3	1	1—2	2—3	1	0	0	0
A.18	6	1	3	6	1	3	3	3	0	1	3	2	1	3	2	0	0	0
	3	1	6	3	1	6	3	3	0	2	3	1	2	3	1	0	0	0
	6	3	1	6	3	2	3	3	1/III	3	1	2	3	2	1	0	0	1
	6	1	3	6	1	3	3	3	0	1	3	2	1	3	2	0	0	0
	3	6	1	1	3	6	3	3	2/I, 3/II, 1/III	3	2	1	2	1	3	2	3	1
A.25	6	1	1	6	1	1	2	2	0	1	1	0	1	1	0	0	0	0
	6	3	1	6	3	1	3	3	0	3	1	2	3	1	2	0	0	0
	6	2	1	6	2	1	3	3	0	2	1	1	2	1	1	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	1	2	6	1	2	6	3	3	0	1	1	2	1	1	2	0	0	0
A.29	4	3	1	4	3	1	3	3	0	1	3	2	1	3	2	0	0	0
	1	1	3/4	1	1	3/4	2	2	0	0	2—3	2—3	0	2—3	2—3	0	0	0
	1	3	3	1	3	3	2	2	0	2	2	0	2	2	0	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	3	4	6	3	4	6	3	3	0	1	3	2	1	3	2	0	0	0

In April I analyzed 25 gonads with 50 testicles and 150 follicles (75 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases, as follows:

Phase / Follicles	1	1/2	2	3	3/4	4	Total follicles	SPERMIOGENESIS 5/6	SPERMIOGENESIS 6	SPERMIOGENESIS Total follicles	Total general
I	9	—	1	7	2	4	23	1	26	27	50
II	18	2	7	14	—	2	43	2	5	7	50
III	18	—	5	12	2	2	39	—	11	11	50
Total follicles	45	2	13	33	4	8	105	3	42	45	150
%	30.00	1.33	8.67	22.00	2.67	5.33	70.00	2.00	28.00	30.00	100%

2. Number of phases presented by the follicles of a testicle: a) a single testicle (2%) present its 3 follicles in the same phase; b) 10 testicles (20%) have follicles in two different phases; c) 39 testicles (78%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	8 follicles = 10.67%	5 follicles = 6.67%
— asynchronism of 1/2 phase	=	1 follicle = 1.33%	1 follicle = 1.33%
— asynchronism of 1 phase	=	24 follicles = 32.00%	25 follicles = 33.33%
— asynchronism of 1—1/2 phase	=	4 follicles = 5.33%	4 follicles = 5.33%
— asynchronism of 2 phases	=	20 follicles = 26.67%	20 follicles = 26.67%
— asynchronism of 2—1/2 phases	=	3 follicles = 4.00%	5 follicles = 6.67%
— asynchronism of 3 phases	=	15 follicles = 20.00%	15 follicles = 20.00%
Total		75 follicles = 100%	75 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 69 pairs (92%) are synchronous: 6 pairs (8%) present the following asynchronism: 3 cases (4%) with 1 phase (1/II and 2/III); 1 case (1.33%) with 2 phases (1/II); 1 case (1.33%) with 2—1/2 phase (1/I); 1 case with 3 phases (1/II). The frequency of asynchronisms is: 2/I; 2/II; 2/III.

Table 5

The structural and functional evolution of the testicular follicles in May

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of sinchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
May 8	3	4	1	3	4	1	3	3	0	1	2	3	1	2	3	0	0	0
	2	1	4	2	1	4	3	3	0	1	2	3	1	2	3	0	0	0
	2	1	4	1	1	3/4	3	2	1/I, 0—1/III	1	2	3	0	2—3	2—3	1	0	0—1
	3	1	4	3	1	4	3	3	0	2	1	3	2	1	3	0	0	0
	3	1	4	3	1	4	3	3	0	2	1	3	2	1	3	0	0	0
May 9	5	3	4	5	3	4	3	3	0	2	1	1	2	1	1	0	0	0
	5	4	2	3	4	5	3	3	2/I, 3/III	2	3	2	1	2	1	2	0	3
	1	3	5	3	1	5	3	3	2/I, 2/II	2	2	2	2	2	2	2	2	0
	4	5	3	4	5	3	3	3	0	1	1	2	1	1	2	0	0	0
May 13	4	3	1	4	3	1	3	3	0	1	3	2	1	3	2	0	0	0
	1	1	3/4	1	1	3	2	2	0—1/III	0	2—3	2—3	0	2	2	0	0	0—1
	1	1	3	1	1	3	2	2	0	0	2	2	0	2	2	0	0	0
	5	3	1	5	3	1	3	3	0	2	2	2	2	2	2	0	0	0
	5	4	3	5	4	3	3	3	0	1	2	1	1	2	1	0	0	0
May 20	6	5	3	6	5	3	3	3	0	1	3	2	1	3	2	0	0	0
	2	3	5	2	3	5	3	3	0	1	3	2	1	3	2	0	0	0
	1	3	6	1	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	5	5/6	3	4/5	5/6	3	3	3	0—1/I	0—1	2	2—3	1	1—2	2—3	0—1	0	0
	4/5	3	5/6	5	3	5/6	3	3	0—1/I	1—2	1	2—3	2	0—1	2—3	0—1	0	0
May 28	6	3	6	6	3	6	2	2	0	3	0	2	3	0	3	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	6	6	4	6	6	2	2	2	2	2	0	2	2	0	0	0	0
	3/4	6	6	4	6	6	2	2	0—1/I	2—3	2—3	0	2	2	0	0—1	0	0

In May I analyzed 24 gonads with 48 testicles and 144 follicles (72 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases, as follows:

Phase								SPERMIOGENESIS				Total general
Follicles	1	2	3	3/4	4	4/5	Total follicles	5	5/6	6	Total follicles	
I	7	6	8	1	11	2	35	9	—	4	13	48
II	13	—	15	—	6	—	34	4	2	8	14	48
III	6	1	12	2	8	—	29	5	2	12	19	48
Total follicles	26	7	35	3	25	2	98	18	4	24	46	144
%	18.06	4.86	24.31	2.08	17.36	1.39	68.06	12.50	2.78	16.66	31.94	100%

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 15 testicles (31.25%) have follicles in two different phases; c) 33 testicles (68.75%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	7 follicles = 9.72%	8 follicles = 11.11%
— asynchronism of 1/2 phase	=	1 follicle = 1.39%	1 follicle = 1.39%
— asynchronism of 1 phase	=	16 follicles = 22.22%	17 follicles = 23.61%
— asynchronism of 1—1/2 phase	=	1 follicle = 1.39	1 follicle = 1.39%
— asynchronism of 2 phases	=	29 follicles = 40.28%	31 follicles = 43.50%
— asynchronism of 2—1/2 phases	=	6 follicles = 8.33%	4 follicles = 5.56%
— asynchronism of 3 phases	=	12 follicles = 16.67%	10 follicles = 13.89%
Total		72 follicles = 100%	72 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 62 pairs (86.11%) are synchronous; 10 pairs (13.89%) present the following asynchronism: 5 cases (6.94%) with 1/2 phase (3/I), 2/III); 1 case (1.39%) with 1 phase (1/I); 3 cases (4.17%) with 2 phases (2/I, 1/II); 1 case (1.39%) with 3 phases (1/III). The frequency of asynchronisms: 6/I; 1/II; 3/III.

Table 6

The structural and functional evolution of the testicular follicles in June

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of sinchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
June 3	1	2	3	1	2	3	3	3	0	1	2	1	1	2	1	0	0	0
	5/6	6	6	5	6	6	2	2	0—1/I	0—1	0—1	0	1	1	0	0—1	0	0
	5/6	6	6	5/6	6	6	2	2	0	0—1	0—1	0	0—1	0—1	0	0	0	0
	6	6	6	6	6	6	1	1	0	0	0	0	0	0	0	0	0	0
	4	6	5/6	4	6	5/6	3	3	0	2	1—2	0—1	2	1—2	0—1	0	0	0
	4	6	5/6	4	6	5/6	2	2	0	2	1—2	0—1	2	1—2	0—1	0	0	0
June 10	3/4	6	5/6	3/4	6	5/6	3	3	0	2—3	2	0—1	2—3	2	0—1	0	0	0
	4	6	5	4	6	5	3	3	0	2	1	1	2	1	1	0	0	0
	3	5/6	4	3	5/6	4	3	3	0	2—3	1	1—2	2—3	1	1—2	0	0	0
	6	5/6	4	6	5/6	4	3	3	0	0—1	2	1—2	0—1	2	1—2	0	0	0
	5	6	6	5	6	6	2	2	0	1	1	0	1	1	0	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
June 20	4	3	6	4	3	6	3	3	0	1	2	3	1	2	3	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
	5	3	6	5	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	6	6	3	6	5/6	3	2	2	0—1/II	0	3	3	0—1	3	2—3	0	0—1	0
	4	6	5/6	3/4	6	5	3	3	0—1/I, 0—1/III	2	1—2	0—1	2—3	1—2	1	0—1	0	0—1
June 26	5/6	4	6	5/6	4	6	2	2	0	1—2	0—1	2	1—2	0—1	2	0	0	0
	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	6	5	4	6	5	4	3	3	0	1	2	1	1	2	1	0	0	0
	6	5/6	5	6	5/6	5	2	2	0	0—1	1	0—1	0—1	1	0—1	0	0	0
	4/5	6	5/6	4	6	5/6	3	3	0—1/I	1—2	1	0—1	2	1—2	0—1	0—1	0	0

In June I analyzed 24 gonads with 48 testicles and 144 follicles (72 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases, as follows:

Phase								SPERMIOGENESIS				
Follicles	1	2	3	3/4	4	4/5	Total follicles	5	5/6	6	Total follicles	Total general
I	2	—	8	3	10	1	24	5	5	14	24	48
II	—	2	4	—	2	—	8	2	7	31	40	48
III	2	—	4	—	8	—	14	7	9	18	34	48
Total follicles	4	2	16	3	20	1	46	14	21	63	98	144
%	2.78	1.39	11.10	2.08	13.89	0.70	31.94	9.73	14.58	43.75	68.06	100%

2. Number of phases presented by the follicles of a testicle: a) 2 testicles have all the follicles in the same phases; b) 22 testicles (45.83%) have follicles in two different phases; c) 24 testicles (50%) have follicles in three different phases.

3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	11 follicles = 15.28%	10 follicles = 13.89
— asynchronism of 1/2 phase	=	13 follicles = 18.06%	11 follicles = 15.28%
— asynchronism of 1 phase	=	16 follicles = 22.22%	18 follicles = 25.00%
— asynchronism of 1—1/2 phase	=	7 follicles = 9.72%	7 follicles = 9.72%
— asynchronism of 2 phases	=	14 follicles = 19.44%	14 follicles = 19.44%
— asynchronism of 2—1/2 phases	=	2 follicles = 2.78%	4 follicles = 5.56%
— asynchronism of 3 phases	=	9 follicles = 12.50%	8 follicles = 11.11%
Total		72 follicles = 100%	72 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 67 pairs (93.06%) are synchronous; 5 pairs (6.94%) present the following asynchronism: 5 cases with 1/2 phase (3/I, 1/II, 1/III).

Table 7

The structural and functional evolution of the testicular follicles in July

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
July 5	4	3	6	4	3	6	3	3	0	1	2	3	1	2	3	0	0	0
	5	3	6	5	2	6	3	3	I/II	2	1	3	3	1	2	0	1	0
	4	6	5	4	6	5	3	3	0	2	1	1	2	1	1	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
July 9	6	5/6	4	6	5/6	4	3	3	0	0–1	2	1–2	0–1	2	1–2	0	0	0
	6	5/6	3	6	5/6	3	2	2	0	0–1	3	2–3	0–1	3	2–3	0	0	0
	4	6	5	4	6	5	3	3	0	2	1	1	2	1	1	0	0	0
	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	2	6	4	2	6	4	3	3	0	2	2	2	2	2	2	0	0	0
	6/1	6	3	6/1	6	3	3	3	0	0–1	2–3	3	0–1	2–3	3	0	0	0
July 18	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
	5	3	5/6	5	3	5/6	2	2	0	2	0–1	2–3	2	0–1	2–3	0	0	0
	5	5	6	5	5	6	2	2	0	0	1	1	0	1	1	0	0	0
	6	5	6	6	4	6	2	2	I/II	1	0	1	2	0	2	0	1	0
July 25	5	3	6	5	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
	6	6	6	6	6	6	1	1	0	0	0	0	0	0	0	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
July 30	6	6	5	6	6	5	2	2	0	0	1	1	0	1	1	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0

In July I analyzed 27 gonads with 54 testicles and 162 follicles (81 pairs).

Conclusions.

1. The follicles are in all the spermatogenetic phases, except phase 1, having the following distribution:

Phase					SPERMIOGENESIS					
Follicles	2	3	4	Total follicles	5	5/6	6	6/1	Total follicles	Total general
I	2	2	16	20	8	—	24	2	34	54
II	1	11	1	13	3	4	34	—	41	54
III	—	4	14	18	8	2	26	—	36	54
Total follicles	3	17	31	51	19	6	84	2	111	162
%	1.85	10.49	19.14	31.48	11.73	3.70	51.85	1.24	68.52	100%

2. Number of phases presented by the follicles of a testicle: a) 2 testicles (3.70%) have all follicles in the same phase; b) 34 testicles (62.96%) have follicles in two different phases; c) 18 testicles (33.34%) have follicles in three different phases.

3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	18 follicles = 22.22%	18 follicles = 22.22%
— asynchronism of 1/2 phase	=	4 follicles = 4.94%	4 follicles = 4.94%
— asynchronism of 1 phase	=	14 follicles = 17.28%	12 follicles = 14.81%
— asynchronism of 1—1/2 phase	=	1 follicle = 1.24%	1 follicle = 1.24%
— asynchronism of 2 phases	=	31 follicles = 38.27%	33 follicles = 40.74%
— asynchronism of 2—1/2 phases	=	3 follicles = 3.70%	3 follicles = 3.70%
— asynchronism of 3 phases	=	10 follicles = 12.35%	10 follicles = 12.35%
Total		81 follicles = 100%	81 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 79 pairs (97.53%) are synchronous; 2 pairs (2.47%) present an asynchronism of 1 phase (2/II).

Table 8

The structural and functional evolution of the testicular follicles in August

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
Aug. 5	6	4	6	6	4	6	2	2	0	2	0	2	2	0	2	0	0	0
	6	6	5	6	6	5	2	2	0	0	1	1	0	1	1	0	0	0
	5	6	5/6	5	6	5/6	2	2	0	1	0–1	0–1	1	0–1	0–1	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
	5/6	4	6	5/6	4	6	3	3	0	1–2	0–1	2	1–2	0–1	2	0	0	0
	5	6	6	5	6	6	2	2	0	1	1	0	1	1	0	0	0	0
Aug. 13	6	5	3	6	5	3/4	3	3	0–1/III	1	3	2	1	3	2	0	0	0–1
	3	5	5	3	5	5	2	2	0	2	2	0	2	2	0	0	0	0
	5	4	6	5	4	6	3	3	0	1	1	2	1	1	2	0	0	0
	4	5/6	6	4	5/6	6	2	2	0	1–2	2	0–1	1–2	2	0–1	0	0	0
	5	5/6	6	5	6	6	3	2	0–1/II	0–1	1	0–1	1	1	0	0	0–1	0–1
Aug. 19	3/4	6	5	3	4	6/5	3	3	0	2–3	1–2	1	2–3	1–2	1	0	0	0
	5	3	6	5	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	5/6	5	6	5	5/6	6	2	2	0	0–1	1	0–1	0–1	1	0–1	0	0	0
	5	5/6	3	5/6	5	3	3	3	0	0–1	2–3	2	0–1	2–3	2	0	0	0
	5	5/6	6	5	5	5/6	2	2	0–1/III, 0–1	0–1	1	0–1	0	0–1	0–1	0	0–1	0–1
Aug. 27	4	5/6	6	4	5/6	6	3	3	0	1–2	2	0–1	1–2	2	0–1	0	0	0
	6	4/5	5/6	6	4/5	5/6	2	2	0	1–2	0–1	1	1–2	0–1	1	0	0	0
	6	6	5	6	6	5	2	2	0	0	1	1	0	1	1	0	0	0
	5	6	6	5	6	5/6	2	2	0–1/III	1	1	0	1	0–1	0–1	0	0	0–1
	6	5/6	5/6	6	5/6	5/6	2	2	0	0–1	0–1	0	0–1	0–1	0	0	0	0

In August I analiyzed 21 gonads with 42 testicles and 126 follicles (63 pairs).

Conclusions.

1. The follicles are in all the spermatogenetic phases except phases 1 and 2, having the following distribution:

Phase						SPERMIOGENESIS				
Follicles	3	3/4	4	4/5	Total follicles	5	5/6	6	Total follicles	Total general
I	2	2	4	—	8	16	4	14	34	42
II	2	—	6	2	10	7	10	15	32	42
III	3	1	2	—	6	8	8	20	36	42
Total follicles	7	3	12	2	24	31	22	49	102	126
%	5.56	2.38	9.52	1.59	19.05	24.60	17.46	38.89	80.95	100%

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 27 testicles (64.29%) have follicles in two different phases; c) 15 testicles (35.71%) have follicles in three different phases.

3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	8 follicles = 12.70%	9 follicles = 14.29%
— asynchronism of 1/2 phase	=	15 follicles = 23.81%	15 follicles = 23.81%
— asynchronism of 1 phase	=	18 follicles = 28.57%	17 follicles = 26.98%
— asynchronism of 1—1/2 phase	=	5 follicles = 7.94%	5 follicles = 7.94%
— asynchronism of 2 phases	=	13 follicles = 20.64%	13 follicles = 20.64%
— asynchronism of 2—1/2 phases	=	2 follicles = 3.17%	2 follicles = 3.17%
— asynchronism of 3 phases	=	2 follicles = 3.17%	2 follicles = 3.17%
Total		63 follicles = 100%	63 follicles = 100%

Degree of phase synchronism and asynchronism among the homologous follicles: 58 pairs (92.06%) are synchronous; 5 pairs 7.94%) present the following asynchronism: with 1/2 phase (2/II and 3/III).

Table 9

The structural and functional evolution of the testicular follicles in September

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
Sept. 3	3	5	5	3	5	4	2	3	1/III	2	2	0	2	1	1	0	0	0
	5	6	5	5	5/6	6	2	2	0—1/II, 1/III	1	0	1	1	0—1	0—1	0	0—1	1
	6	5/6	3	6	5/6	3	3	3	0	0—1	3	2—3	0—1	3	2—3	0	0	0
	6	4	6	6	4	6	2	2	0	2	0	2	2	0	2	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
Sept. 9	6	3	5	6	3	5	3	3	0	3	1	2	3	1	2	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	5/6	3	6	5/6	3	6	3	3	0	2—3	0—1	3	2—3	0—1	3	0	0	0
	6	6	4	6	6	4	2	2	0	0	2	2	0	2	2	0	0	0
	4/5	6	6	4/5	6	6	2	2	0	2—1	2—1	0	2—1	2—1	0	0	0	0
Sept. 10	3	6	5	3	6	5/6	3	3	0—1/III	3	2	1	3	2—3	0—1	0	0	0—1
	4	6	5	4	6	6	3	2	1/III	2	1	1	2	2	0	0	0	1
	4/5	6	5	4/5	6	6	3	2	1/III	2—1	0—1	1	2—1	2—1	0	0	0	1
	6	5	3	6	5	3	3	3	0	1	3	2	1	3	2	0	0	0
	5	6	6	5	6	6	2	2	0	1	1	0	1	1	0	0	0	0
Sept. 17	4	6	5	4	6	5	3	3	0	2	1	1	2	1	1	0	0	0
	5/6	5	3	5/6	5	3	2	2	0	0—1	2—3	2	0—1	2—3	2	0	0	0
	4	5/6	5	4	5/6	5	2	2	0	1—2	1	0—1	1—2	1	0—1	0	0	0
	4	4	5/6	4	4	5/6	2	2	0	0	1—2	1—2	0	1—2	1—2	0	0	0
	5/6	5	4	5/6	5	4	2	2	0	0—1	1—2	1	0—1	1—2	1	0	0	0
Sept. 23	4	6	5/6	4	6	5/6	3	3	0	2	1—2	0—1	2	1—2	0—1	0	0	0
	5/6	6	4	6	6	5	3	2	0—1/I, 1/III	0—1	1—2	2	0	1	1	0—1	0	1
	5/6	4	6	5/6	4	6	3	3	0	1—2	0—1	2	1—2	0—1	2	0	0	0
	5	5	3	5/6	5	3	2	2	0—1/I	0	2	2	0—1	2—3	2	0—1	0	0
	6	5	4	6	5	4	3	3	0	1	2	1	1	2	1	0	0	0

Sept.	4	5	5	4	5	5	2	2	0	1	1	0	1	1	0	0	0	0
27	5	3	6	5	3	6	3	3	0	2	1	3	2	1	3	0	0	0
	6	4	3	6	4	3	3	3	0	2	3	1	2	3	1	0	0	0
	5	4	6	5	4	6	3	3	0	1	1	2	1	1	2	0	0	0
	6	5	4/5	6	5	4	3	3	0—1/III	1	1—2	0—1	1	2	1	0	0	0—1

In September I analyzed 30 gonads with 60 testicles and 180 follicles (90 pairs).

Conclusions.

1. The follicles are in all the spermatogenetic phases, except phase 1 and 2, as follows:

Phase	SPERMIOGENESIS								
Follicles	3	4	4/5	Total follicles	5	5/6	6	Total follicles	Total general
I	4	14	4	22	9	10	19	38	60
II	8	10	—	18	16	5	21	42	60
III	10	9	1	20	14	5	21	40	60
Total follicles	22	33	5	60	39	20	61	120	180
%	12.23	18.33	2.77	33.34	21.67	11.12	33.88	66.66	100%

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 30 testicles (50%) have follicles in two different phases; c) 30 testicles (50%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III():

		left	right
— synchronism	=	11 follicles = 12.22%	12 follicles = 12.22%
— asynchronism of 1/2 phase	=	10 follicles = 11.11%	11 follicles = 11.11%
— asynchronism of 1 phase	=	23 follicles = 25.56%	23 follicles = 25.56%
— asynchronism of 1—1/2 phase	=	11 follicles = 12.22%	10 follicles = 11.11%
— asynchronism of 2 phases	=	23 follicles = 25.56%	21 follicles = 23.33%
— asynchronism of 2—1/2 phases	=	3 follicles = 3.33%	5 follicles = 5.56%
— asynchronism of 3 phases	=	9 follicles = 10.00%	9 follicles = 10.00%
Total		90 follicles = 100%	90 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 80 pairs (89%) are synchronous; 10 pairs (11%) present the following asynchronism: 5 cases with 1/2 phase (2/I, 2/III, 1/II); 5 cases with 1 phase (5/III).

Table 10

The structural and functional evolution of the testicular follicles in October

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
Oct. 1	5	4	6	5	4	6	3	3	0	1	1	2	1	1	2	0	0	0
	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	3	5	5	3	6	5	2	3	1/II	2	2	0	3	2	1	0	1	0
	5	6	6	5	6	6	2	2	0	1	1	0	1	1	0	0	0	
	6	5	5	6	5	5	2	2	0	1	1	0	1	1	0	0	0	0
Oct. 4	3	5	1	3/4	5/6	1	3	3	0—1/I, 0—1/II	2	2	2	2	2	1	0—1	0—1	0
	5	6	1	5	6	1	3	3	0	1	2	1	1	2	1	0	0	0
	1	6	5	1	6	5	3	3	0	1	2	1	1	2	1	0	0	0
	5	6	4	5	6	4	3	3	0	1	1	2	1	1	2	0	0	0
	6	1	6	6	1	6	2	2	0	1	0	1	1	0	1	0	0	0
Oct. 10	6	5/6	5/6	6	5/6	5/6	2	2	0	0—1	0—1	0	0—1	0—1	0	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	6	6	4	6	6	6	2	1	2/III	0	2	2	0	0	0	0	0	2
	6	4	3	6	4	3/4	3	3	0—1/III	2	3	1	2	2—3	0—1	0	0	0—1
	6	2	6	6	2	6	2	2	0	2	0	2	2	0	2	0	0	0
Oct. 14	6	1	6	6	1	6	2	2	0	1	0	1	1	0	1	0	0	0
	4/5	6	6	4/5	6	6	2	2	0	1—2	1—2	0	1—2	1—2	0	0	0	0
	6	6	3	6	6	3	2	2	0	0	3	3	0	3	3	0	0	0
	6	5	3	6	5	3	3	3	0	1	3	2	1	3	2	0	0	0
	3	6	6	3/4	6	6	2	2	0—1/I	3	3	0	2—3	2—3	0	0—1	0	0
Oct. 21	6	4	6	6	4	6	2	2	0	2	0	2	2	0	2	0	0	0
	1	6	6	1	6	6	2	2	0	1	1	0	1	1	0	0	0	0
	6	6	3/4	6	6	4	2	2	0—1/III	0	2—3	2—3	0	2	2	0	0	0—1
	4/5	1	6	4/5	1	6	3	3	0	2—3	1—2	1	2—3	1—2	1	0	0	0
	1	6	6	4	6	6	2	2	3/I	1	1	0	2	2	0	3	0	0

Oct.	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
28	6	6	6	6	6	6	1	1	0	0	0	0	0	0	0	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
	4/5	6	6/1	4/5	6	6/1	3	3	0	1—2	2—3	0—1	1—2	2—3	0—1	0	0	0

In October I analyzed 30 gonads with 60 testicles and 180 follicles (90 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases, having the following distribution:

Phase	SPERMIOGENESIS												
Follicles	1	2	3	3/4	4	4/5	Total follicles	5	5/6	6	6/1	Total follicles	Total general
I	5	—	8	2	1	6	22	8	—	30	—	38	60
II	6	2	2	—	6	—	16	6	3	35	—	44	60
III	8	—	5	2	4	—	19	8	2	29	2	41	60
Total follicles	19	2	15	4	11	6	57	22	5	94	2	123	180
	10.55	1.11	8.33	2.22	6.12	3.33	31.66	12.23	2.77	52.23	1.11	68.34	100%

2. Number of phases presented by the follicles of a testicle: a) 3 testicles (5%) have all the follicles in the same phase; b) 36 testicles (60%) have follicles in two different phases; c) 21 testicles (35%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	22 follicles = 24.44%	23 follicles = 25.56%
— asynchronism of 1/2 phase	=	3 follicles = 3.34%	4 follicles = 4.44%
— asynchronism of 1 phase	=	28 follicles = 31.11%	27 follicles = 30.00%
— asynchronism of 1—1/2 phase	=	4 follicles = 4.44%	4 follicles = 4.44%
— asynchronism of 2 phases	=	18 follicles = 20.00%	18 follicles = 20.00%
— asynchronism of 2—1/2 phases	=	4 follicles = 4.44%	5 follicles = 5.56%
— asynchronism of 3 phases	=	11 follicles = 12.23%	9 follicles = 10.00%
Total		90 follicles = 100%	90 follicles = 100%

4. Degree of phase synchronism and asynchronism among th ehomologous follicles: 82 pairs (91%) are synchronous; 8 pairs (9%) present the following asynchronism: 5 cases with 1/2 phase (2/I, 1/II, 2/III); 1 case with 1 phase (1/II); 1 case with 2 phases (2/III); 1 case with 3 phases (1/I).

Table 11

The structural and functional evolution of the testicular follicles in November

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicle		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
Nov. 5	6	6/1	1	6	6/1	1	2	2	0	0—1	1	0—1	0—1	1	0—1	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	6	6	4	6	6	2	2	0	2	2	0	2	2	0	0	0	0
	4	2	6	4	2	6	3	3	0	2	2	2	2	2	2	0	0	0
	4	6	6	4	2	6	2	3	2/II	2	2	0	2	2	2	0	2	0
Nov. 11	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
	6	2	6	6	2	6	2	2	0	2	0	2	2	0	2	0	1	0
	6	6	3	6	6	3	2	2	0	0	3	3	0	3	3	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
	6	4	6	6	4	6	2	2	0	2	0	2	2	0	2	0	0	0
Nov. 13	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
Nov. 19	6	4/1	6	6	4/1	6	2	2	0	2—3	0	2—3	2—3	0	2—3	0	0	0
	6	3	6	6	3	6	2	2	0	3	0	3	3	0	3	0	0	0
	6	6	5	6	1	6	2	2	1/II, 1/III	0	1	1	1	0	1	0	1	1
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
Nov. 26	6	6	1	2	6	1	2	3	2/I	0	1	1	2	1	1	2	0	0
	2	6	1	6	2	6	3	2	2/I, 2/II	2	1	1	2	0	2	2	2	1
	6	1	3	6	1	3	3	3	0	1	3	2	1	3	2	0	0	0
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
	1	6	1	1	6	1	2	2	0	1	0	1	1	0	1	0	0	0

In November I analyzed 21 gonads with 42 testicles and 126 follicles (63 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases having the following distribution:

Phase							SPERMIOGENESIS				
Follicles	1	2	3	4	4/1	Total follicles	5	6	6/1	Total follicles	Total general
I	2	2	—	8	—	12	—	30	—	30	42
II	3	6	8	2	2	21	—	19	2	21	42
III	13	—	4	—	—	17	1	24	—	25	42
Total follicles	18	8	12	10	2	50	1	73	2	76	126
%	14.28	6.34	9.53	7.94	1.59	39.68	0.80	57.93	1.59	60.32	100%

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 35 testicles (83.33%) have follicles in two different phases; c) 7 testicles (16.67%) have follicles in three different phases.

3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II and III):

		left	right
— synchronism	=	17 follicles = 26.98%	16 follicles = 25.40%
— asynchronism of 1/2 phase	=	2 follicles = 3.18%	2 follicles = 3.18%
— asynchronism of 1 phase	=	16 follicles = 25.40%	14 follicles = 22.22%
— asynchronism of 2 phases	=	15 follicles = 23.80%	18 follicles = 28.56%
— asynchronism of 2—1/2 phases	=	2 follicles = 3.18%	2 follicles = 3.18%
— asynchronism of 3 phases	=	11 follicles = 17.46%	11 follicles = 17.46%
Total		63 follicles = 100%	63 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 56 pairs (89%) are synchronous; 7 pairs (11%) present the following asynchronism: 3 cases with 1 phase (1/II, 2/III); 4 cases with 2 phases (2/I, 2/II).

Table 12

The structural and functional evolution of the testicular follicles in December

Date	Phase of germinal element development in follicles I, II, III						Number of phases in the follicles of a testicles		Phase difference between the two testicles	Degree of synchronism and asynchronism between the phases of the 3 follicles (I-II, I-III, II-III)						Degree of synchronism and asynchronism between the phases of the homologous follicles of the two testicles		
	left			right						left			right					
	I	II	III	I	II	III	left	right								I	II	III
Dec. 3	3	6	1	3	6/1	1	3	3	0—1/II	3	2	1	3	2	0—1	0	0—1	0
	1	6	1	6/1	6	1	2	2	0—1/I	1	0	1	0—1	0—1	1	0—1	0	0
	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	1	6	6	1	6	6	2	2	0	1	1	0	1	1	0	0	0	0
Dec. 13	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
	6	5	3	6	5	3	3	3	0	1	3	2	1	3	2	0	0	0
	6	5	3	6	5	3	3	3	0	1	3	2	1	3	2	0	0	0
	3	1	2	3	6/2	2	3	3	0—1/II	2	1	1	1—3	1	2—0	0	1	0
	5	1	6	3	6	6—1	3	3	2/I, 0—1/III	2—3	1—2	1	3	2—3	0—1	2	1	0—1
Dec. 20	6	1	4	6	1	4	3	3	0	1	2	3	1	2	3	0	0	0
	3	5	3	3	3	6	2	2	2/II	2	0	2	0	3	3	0	2	3
	1	1	6	1	1	2	2	2	2/III	0	1	1	0	1	1	0	0	2
	1	6	6/2	1	6	6/2	3	3	0	1	1	0—2	1	1	0—2	0	0	0
	3	6	6	3	6	6	2	2	0	3	3	0	3	3	0	0	0	0
Dec. 27	3	6	5	3	6	5	3	3	0	3	2	1	3	2	1	0	0	0
	3	6	5/6	3	6	5/6	2	2	0	3	2/3	0—1	3	2—3	0—1	0	0	0
	3	6	4	3	6	4	3	3	0	3	1	2	3	1	2	0	0	0
	6	6	1	6	6	1	2	2	0	0	1	1	0	1	1	0	0	0
	6	6	1	6	6/1	1	2	2	0—1/II	0	1	1	0—1	1	0—1	0	0—1	0

In December I analyzed 20 gonads with 40 testicles and 120 follicles (60 pairs).

Conclusions.

1. The follicles present all the spermatogenetic phases, having the following distribution:

Phase	SPERMIOGENESIS											
Follicles	1	2	3	4	Total follicles	5	5/6	6	6/1	6/2	Total follicles	Total general
I	7	—	21	—	28	1	—	10	—	—	12	40
II	6	—	1	—	7	5	—	25	2	1	33	40
III	8	3	5	4	20	4	2	11	1	2	20	40
Total follicles	21	3	27	4	55	10	2	46	4	3	65	120
%	17.50	2.50	22.50	3.33	45.83	8.34	1.66	38.34	3.33	2.50	54.17	100%

2. Number of phases presented by the follicles of a testicle: a) no testicle has all the follicles in the same phases; b) 20 testicles (50%) have follicles in two different phases; c) 20 testicles (50%) have follicles in three different phases.
3. Degree of phase synchronism and asynchronism among the follicles of the same testicles (I, II, III):

		left	right
— synchronism	=	9 follicles = 15.00%	7 follicles = 11.66%
— asynchronism of 1/2 phase	=	1 follicle = 1.70%	7 follicles = 11.66%
— asynchronism of 1 phase	=	23 follicles = 38.32%	17 follicles = 28.34%
— asynchronism of 2 phases	=	13 follicles = 21.65%	12 follicles = 20.00%
— asynchronism of 3 phase	=	14 follicles = 23.33%	17 follicles = 28.34%
Total		60 follicles = 100%	60 follicles = 100%

4. Degree of phase synchronism and asynchronism among the homologous follicles: 50 pairs (83.35%) are synchronous; 10 pairs (16.65%) present the following asynchronism: 4 cases with 1/2 phase (1/I, 2/II, 1/III); 2 cases with 1 phase (2/II); 3 cases with 2 phases (1/I, 1/II, 1/III); 1 case with 3 phases (1/III).

Correlating these data with the seasonal activity of the androgenic gland [8], I can conclude that this gland presents a maximum activity in April, while the amount of spermatozoa from the follicles (phase 6) decreases abruptly in April-May. This means that the hormonal fraction of RER origin from the androgenic hormonal complex [10] determined the onset of mating in April. Then, the hormonal fraction, synthesized in the Golgi complex, determined the realease of spermatozoa from the follicles, and their transfer to the seminal reservoir. These spermatozoa, together with the ones already deposited in the reservoir, are eliminated and used in the mating and fertilization which occur in April-May. Thus, all mature spermatozoa that have been preserved all winter are used. Just after the mating period, there is an intense spermatogenetic activity, expressed by a transformation of germinal elements of phase 3 into phase 4 and then 5, phases that develop in March and attain a maximum in May (Fig. 5). Also, after mating, an intense spermiogenesis occurs with a maximum production of mature spermatozoa being reached in June. Then, it is maintained at high levels (with small variations) until November, and with this amount of spermatozoa the isopods begin hibernation. The slight drop in the amount of spermatozoa noticed in the second half of August may have been caused either by a short aestival rest, or rather by a new elimination of spermatozoa required by another mating and which helped the mature females deposit a supplemental reserve of spermatozoa to be used in the spring fertilization.

As one can see from the data presented in the synoptic Table 14, the 3 follicles of a gonad do not function synchronously, except for an

Table 14

THE ANNUAL EVOLUTION OF THE SPERMATOGENETIC PHASES OF EACH FOLLICLE (%)

PHASE	1			2			3			4			5			6		
FOLLICLE	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
I	10.80	4.90	8.82	1.96	2.94	2.94	8.80	9.80	3.92	—	1.00	—	1.00	4.94	1.96	10.78	11.75	15.67
II	16.67	9.65	23.17	5.26	0.83	1.76	2.53	2.63	2.63	—	—	—	—	—	—	8.77	20.18	8.77
III	8.33	6.26	8.33	2.08	2.09	—	12.50	10.42	—	—	—	—	6.25	2.08	6.25	4.17	12.50	18.74
IV	6.00	12.00	12.00	0.67	6.00	3.33	4.67	9.33	6.00	4.00	1.33	2.67	—	—	—	18.00	4.67	7.33
V	4.66	9.33	4.17	4.17	—	0.69	5.56	10.42	8.33	8.33	4.17	6.94	7.64	2.78	3.47	2.78	6.94	9.72
VI	1.39	—	1.39	—	1.39	—	5.56	2.77	2.77	9.02	1.39	5.56	4.18	1.39	4.86	13.19	26.39	18.75
VII	—	—	—	1.23	0.62	—	1.23	6.79	2.47	9.88	0.62	8.64	4.94	1.85	4.94	16.05	23.46	17.28
VIII	—	—	—	—	—	—	1.59	1.59	2.38	4.76	4.76	2.38	12.70	7.14	6.35	14.29	19.84	22.22
IX	—	—	—	—	—	—	2.22	4.44	5.57	7.78	5.55	5.00	7.22	8.89	8.33	16.12	14.44	14.44
X	2.78	3.33	4.44	—	1.11	—	4.44	1.11	2.78	1.68	3.33	3.33	7.78	3.33	4.45	16.67	21.11	18.33
XI	1.59	2.38	10.31	1.59	4.75	—	—	6.35	3.18	6.35	3.18	—	—	—	0.80	23.81	16.67	19.04
XII	5.83	5.00	6.67	—	—	2.50	17.50	0.83	4.17	—	—	3.33	0.83	4.17	3.34	9.17	23.33	13.33
MEAN	4.85	4.38	6.30	1.41	1.65	0.95	5.56	5.54	3.85	4.32	2.11	3.15	4.38	2.86	3.73	12.82	16.19	15.30

THE ANNUAL SPERMATOGENETIC CYCLE OF PORCELLIO SCABER LATR.

Table 13

MONTHS	PROCESSED GONADS				THE SPERMATOGENETIC PHASES OF THE FOLLICLES (%)																NUMBER OF PHASES OF THE THREE FOLLICLES (%)			DEGREE OF PHASE SYNCHRONISM AND ASYNCHRONISM AMONG THE FOLLICLES OF THE SAME TESTICLES (%)														DEGREE OF PHASE SYNCHRONISM AND ASYNCHRONISM AMONG THE HOMOLOGOUS FOLLICLES OF THE TESTICLES (%)							THE FREQUENCY OF ASYNCHRONISM PER FOLLICLES (NO.)		
	TOTAL GONADS	TOTAL TESTICLES	TOTAL PAIRS	TOTAL FOLLICLES	1	1/2	1/3	2	2/3	3	3/4	4	4/5	4/1	SPERMIOGENESIS 5	5/1	5/6	6	6/1	6/2	TOTAL (%)	IN ALL IN THE SAME PHASES	IN TWO DIFFERENT PHASES	IN THREE DIFFERENT PHASES	LEFT. SYNCHRONISM	1/2	1	1 1/2	2	2 1/2	3	RIGHT SYNCHRONISM	1/2	1	1 1/2	2	2 1/2	3	SYNCHRONISM	1/2	1	1 1/2	2	2 1/2	3	I	II	III
I	17	34	51	102	24,52	—	1,00	6,84	1,96	20,56	—	1,00	2,94	—	1,00	1,96	1,00	37,22	—	—	41,18	—	30,00	70,00	9,84	—	39,22	—	29,41	—	21,57	11,76	1,96	33,33	—	27,45	—	25,50	86,28	5,88	1,96	1,96	1,96	1,96	—	2	4	1
II	19	38	57	114	46,49	—	1,76	6,14	—	7,89	—	—	0,88	—	—	—	—	34,20	1,76	0,86	36,84	—	65,79	34,21	21,05	8,77	43,86	1,75	15,80	1,75	7,02	21,05	1,75	52,63	—	15,80	—	8,77	82,46	1,75	7,02	—	7,02	1,75	—	2	4	4
III	16	32	48	96	22,92	—	—	4,17	—	22,92	—	—	4,16	—	10,42	—	—	35,41	—	—	45,83	—	37,50	62,50	12,50	2,08	29,17	2,08	31,25	—	22,92	12,50	2,08	29,17	2,08	31,25	—	22,92	100%	—	—	—	—	—	—	0	0	0
IV	25	50	75	150	30,00	1,33	—	8,67	—	22,00	2,67	5,33	—	—	—	—	2,00	28,00	—	—	30,00	2,00	20,00	78,00	10,67	1,33	32,00	5,33	26,67	4,00	20,00	6,67	1,33	33,33	5,33	26,67	6,67	20,00	92%	—	4,00	—	1,33	1,33	1,34	2	2	2
V	24	48	72	144	18,06	—	—	4,86	—	24,31	2,08	17,36	1,39	—	12,50	—	2,78	16,66	—	—	31,94	—	31,25	68,75	5,72	1,39	22,22	1,39	40,28	8,33	16,67	11,11	1,39	23,61	1,39	43,05	5,56	13,89	86,11	6,94	1,39	—	4,17	—	1,39	6	1	3
VI	24	48	72	144	2,78	—	—	1,39	—	11,10	2,08	13,89	0,70		9,73		14,58	43,75	—	—	68,06	4,17	45,83	50,00	15,28	18,06	22,22	9,72	19,44	2,78	12,50	13,89	15,28	25,00	9,72	19,44	5,56	11,11	93,06	6,94	—	—	—	—	—	3	1	1
VII	27	54	81	162	—	—	—	1,85	—	10,49	—	19,14	—	—	11,73	—	3,70	51,85	1,24	—	63,52	3,70	52,96	33,34	22,22	4,94	17,28	1,24	38,27	3,70	12,35	22,22	4,94	14,81	1,24	40,74	3,70	12,35	97,53	—	2,47	—	—	—	—	—	2	—
VIII	21	42	63	126	—	—	—	—	—	5,56	2,38	9,52	1,59	—	24,60	—	12,70	38,89	—	—	83,95	—	64,29	35,71	12,70	3,81	28,57	7,94	20,64	3,17	3,17	14,29	23,81	26,98	7,94	20,64	3,17	3,17	92,06	7,94	—	—	—	—	—	—	2	3
IX	30	60	90	180	—	—	—	—	—	12,23	—	18,33	2,77	—	21,67	—	11,12	33,88	—	—	66,66	—	50,00	50,00	12,22	11,11	25,56	12,22	25,56	3,33	10,00	12,22	12,22	25,56	11,11	23,33	5,56	10,00	89,00	5,50	5,50	—	—	—	—	2	1	7
X	30	60	90	180	10,55	—	—	1,11	—	8,33	2,22	6,12	3,33	—	12,23	—	2,77	32,23	1,11	—	68,34	5,00	60,00	35,00	24,44	3,34	31,11	4,44	20,00	4,44	12,23	25,56	4,44	30,00	4,44	20,00	5,56	10,00	91,00	5,70	1,10	—	1,10	—	1,10	3	2	3
XI	21	42	63	126	14,28	—	—	6,34	—	9,53	—	7,94	—	1,59	0,80	—	—	57,93	1,59	—	60,32	—	63,33	16,67	26,98	3,18	25,40	—	23,80	3,18	17,46	25,40	3,18	22,22	—	28,56	3,18	17,46	89,00	—	4,75	—	6,25	—	—	2	3	2
XII	20	40	60	120	17,50	—	—	2,50	—	22,50	—	3,33	—	—	8,34	—	1,66	38,34	3,33	2,50	54,17	—	50,00	50,00	15,00	1,70	38,32	—	21,65	—	23,33	11,66	11,66	28,34	—	20,00	—	28,34	83,35	6,66	3,33	—	5,00	—	1,66	2	5	3
TOTAL	274	548	822	1644	15,59	0,11	0,23	3,66	0,16	14,78	0,96	8,50	1,48	0,13	9,42	0,16	4,76	39,03	0,75	0,28	54,40	1,24	50,08	48,68	15,05	6,65	29,58	3,84	26,06	2,89	14,93	15,69	7,00	28,75	3,60	26,41	3,26	15,29	90,15	3,94	2,63	0,16	2,24	0,42	0,46	24	27	29
					MEAN % →																																										TOTAL		

unsignificant 1.24% per year. The great majority of follicles are either in two different phases (50.08%), or in 3 different phases (48.68%). This approximately equal ratio holds only for June, September and December. In February, July, August, October and November, most of the follicles are in different phases, while in January, March, April and May, most of the follicles are in 3 different phases, thus demonstrating that the 3 follicles of a gonad function asynchronously most of the year.

Moreover, when comparing the degree of functional synchronism and asynchronism among the 3 follicles of the right hand gonad, it can be noticed that there is a roughly equal ratio between synchronism and asynchronism, which suggests a phase synchronism between the homologous, symmetrical follicles of the two gonads. Indeed, analyzing these follicles I saw that over 90% of them function synchronously, the most frequent degree of phase asynchronism being of half a phase or one phase, at the most. These asynchronisms appear with approximately the same frequence in follicles I (24), II (27) and III (29), and occur mostly during the cold months (see Table 13). The 100% synchronism noticed in March might be caused both by degenerations that took place during hibernation, and by the fact that the spermatogenetic processes have not been reactivated, yet. But, we can say that the homologous follicles of the two symmetrical gonads function synchronously, they being in the same spermatogenetic phase almost all the time.

In order to outline the proportion of follicles in each spermatogenetic phase, I prepared a table with percentage data on the evolution of each spermatogenetic phase in each of the 3 follicles/gonad, for one year (Table 14). The graphs based on the data from this table are relevant for the evolution and monthly amplitude of the spermatogenetic phases undergone by the 3 follicles of each gonad (Figs. 6 and 7).

Phase 1 has an almost parallel evolution in the 3 follicles, with a maximum in February, an interruption in July — September, and a new rise in October — December. The greatest annual amplitude of this phase occurs in follicle III, followed by follicle I and then II.

Phase 2 presents a low amplitude all the year round, and in all the follicles, demonstrating that it occurs in a short period of time. During the first half of the year, the evolution of phase 2 from follicle I is opposite to its evolution in follicles II and III. It is worth mentioning that the lowest annual amplitude of phase 2 can be seen in follicle III. The evolution of this phase is suspended in August — September.

Phase 3 is continuous throughout the year at a moderate level, with a maximum amplitude in March — May in follicles I and II, and again in December in follicle I. Phase 3 presents a minimum in follicle III in March, right when it is at a maximum in follicles I and II. Also, a stagnation of phase 3 in follicle I was observed in November, but the activity suddenly rised to reach its annual maximum in December.

Phase 4 is discontinuous during the year, missing from December till April. In follicles I and III, this phase reaches a maximum amplitude between April and July, while in the same period it is at a minimum in follicle II. Phase 4 presents a second maximum in all the

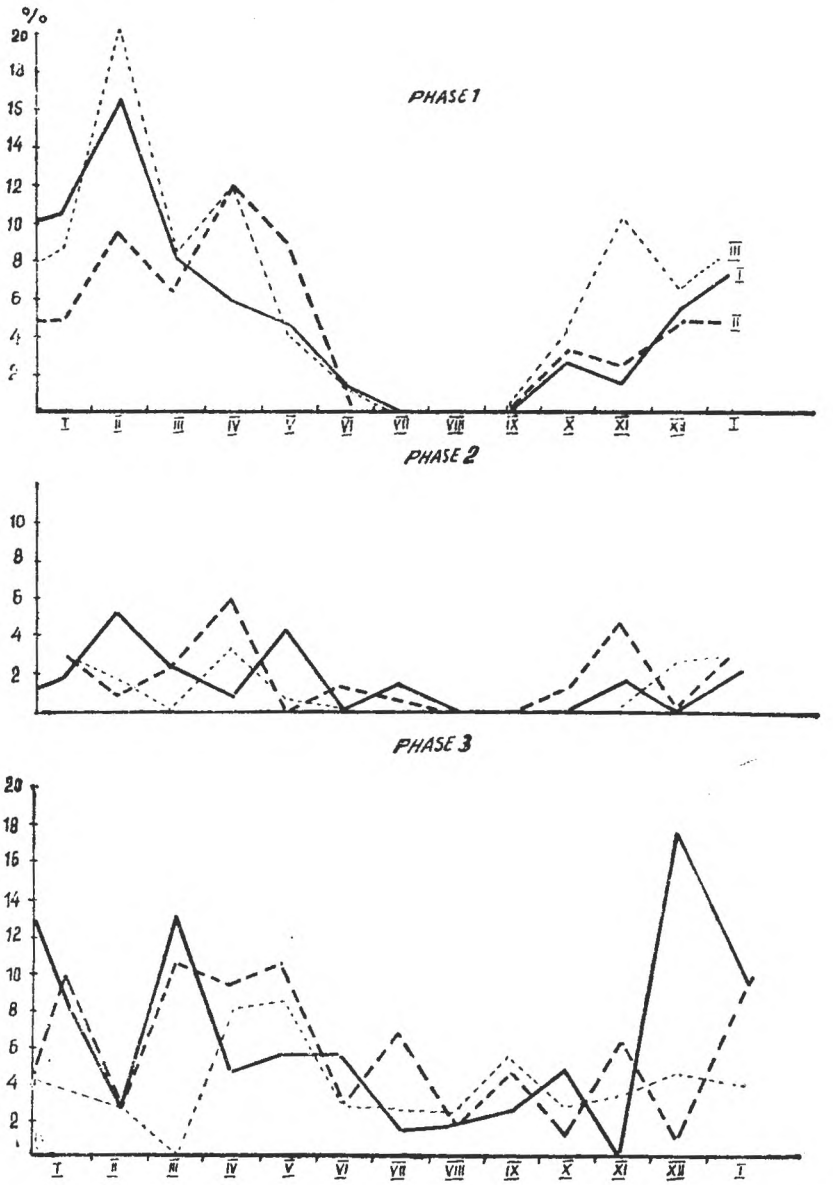


Fig. 6. *Monthly evolution and amplitude of the spermatogenetic phases (1, 2, 3) in follicles I, II and III (%).*

follicles in August — September, and even a third one, in follicle I in November.

Phase 5 shows an almost parallel annual evolution in the 3 follicles, with a peak in March and May, a maximum in August — September,

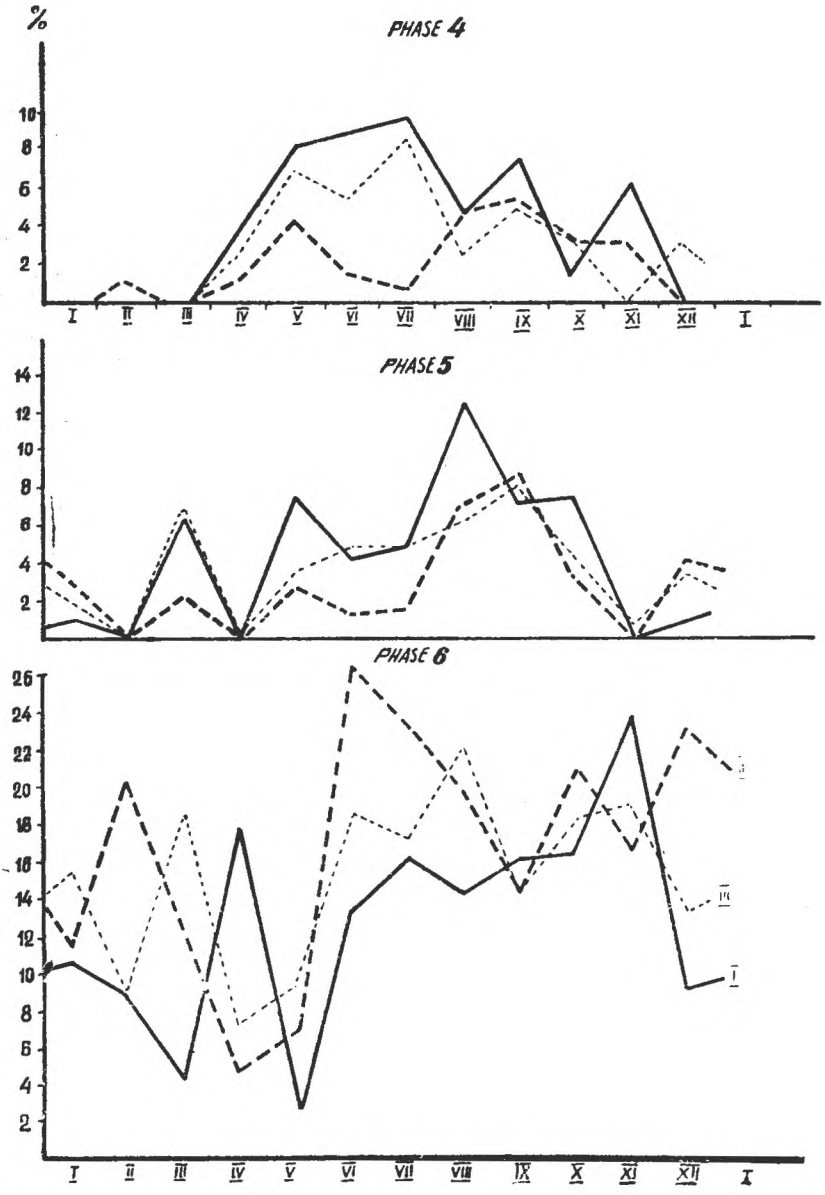


Fig. 7. *Monthly evolution and amplitude of the spermatogenetic phases (4, 5, 6) in follicles I, II and III* (%).

and 3 minima in February, April and November. Follicle II shows a lower annual activity than follicles I and III.

Phase 6 is continuous throughout the year and in all the follicles, but its evolution and amplitude are different for each follicle. Follicle I

presents the lowest activity in this respect, and follicle II the highest. It is interesting that each follicle presents a spring maximum which is distributed in time as follows: follicle II in February, follicle III in March, and follicle I in April, each followed by an immediate decline, *i.e.* by a minimum of activity. This decline indicates that the spermatozoa have gradually moved into the seminal reservoir, first from follicle II, then from follicle III and finally from follicle I, in order to be used in the spring mating which occurs in April — May. After that, the formation of spermatozoa is at a maximum in June when follicle II is most productive. Production of spermatozoa is maintained at a high level until August. Then, it decreases a little in the second half of August and first half of September, and rises again towards the end of the year, when a maximum is reached by follicle I, followed closely by follicle II. This little asynchronism among the 3 follicles ensures an almost continuous production of spermatozoa, similarly to a functional wave.

As one can see from the graphs, the phases which are related, *i.e.* those that follow one another, usually have an antagonistic development, meaning that when one decreases, the next one increases, due to the transformation of the germinal elements of one phase into the germinal elements of the next. Also, from the evolution of each phase, I noticed that one of the follicles always presents a more reduced activity than the other two.

Studying the annual evolution and amplitude of the spermatogenetic phases, one can see that there is a wave of maxima that begins in February with phase 1, continues in March — May with phases 2 and 3, phase 4 in May — July, phase 5 in August — September, and ends with phase 6, which presents a second maximum in October — November.

The spermatogenetic activities are under the influence of the 3 components of the androgenic hormonal complex demonstrated for crustaceans in general [22], and for *Porcellio scaber* in particular [4, 8, 10]. The works of Katakura [23, 24] and Juchault and Legrand [19] have documented in 1961 and 1967 the existence of a hormonal control for the development of the sexual characters and sex differentiation in isopods.

It is considered that the control of all the activities related to the physiology of reproduction in crustaceans and, therefore, in isopods, is a neurohormonal one, directed by the protocerebron [15, 17, 18, 20, 21, 27]. Similar data from other invertebrate groups support this hypothesis and indicate that the neurosecretion of the protocerebron cells, more specifically of *pars intercerebralis*, exerts a regulatory action on the spermatogenetic cycle [11—13].

Conclusions. The data complex obtained through this study demonstrates that in *Porcellio scaber* there is a single spermatogenetic cycle per year, under the climatic conditions of Romania. In this cycle, the evolution of the spermatogenetic phases in the 3 follicles of a gonad shows a functional asynchronism of 2 or 3 phases between the follicles

of the same gonad, but a complete synchronism between the homologous follicles of the two symmetrical gonads.

This conclusion can be extended for the terrestrial isopods from the temperate region whith great seasonal climate variations, where their spermatogenetic cycle occurs just once a year. The dynamic processes of this cycle occur in the warm months of spring, summer and autumn and are separated for the next cycle by a winter diapause (hibernal rest).

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METODE PENTRU IMOBILIZAREA CULTURILOR CELULARE VEGETALE PRODUCĂTOARE DE METABOLIŢI SECUNDARI

CONSTANTIN DELIU*, GABRIELA ISPAS şi CORNELIA MUNTEANU-DELIU****

SUMMARY. — Methods for Immobilization of Plant Cell Cultures Producing Secondary Metabolites. The present pater reviews the researches on the immobilization of plant cells. The production of secondary metabolites using plant cell suspension cultures has been the subject of many researches in the recent years. It was found that many plant cell cultures gradually fail to produce secondary metabolites at levels characteristic of the intact plants. One of the techniques that may diminish or even stop this process is the immobilization of cells. Being immobilized, the cells remain on or in a restricted area. The material in which they are immobilized is mostly known as „matrix". Compared to cell suspensions, the conditions created by immobilization more closely resemble those of the organized plant tissue. The cell/cell contact and the rudimentary differentiation induced by immobilization can be beneficial for secondary metabolite production. The effects of various immobilization strategies on cell physiology always include a decreased growth rate; however, their effects on secondary metabolite biosynthesis are contraversial. Thus, productivity of a plant cell culture may increase or decrease depending on the chemical nature of the matrix on or in which the cells were immobilized.

1. Introducere

Conceptul de imobilizare este cunoscut de mai mult timp sub un alt termen legat de cercetările de enzimologie bacteriană, şi anume acela de „enzime ataşate de o suprafaţă solidă". Realizarea comercială a acestui concept s-a produs însă prin anii 1960 [39].

O altă metodă asemănătoare cu imobilizarea enzimelor este imobilizarea de celule întregi ce etalează activităţi enzimatice dorite, metodă ce elimină timpul şi etapele costisitoare legate de purificarea enzimelor.

Termenul de celule imobilizate, utilizat aici, este definit ca şi un sistem în care celulele sunt ataşate şi obligate să rămână pe sau într-o regiune restrânsă de spaţiu.

Celulele procariote reţin în prezent mai mult atenţia, deoarece se obţin mult mai uşor şi se multiplică fără greutate în condiţii industriale. Totuşi, în ultimul timp, aria acestei tehnici s-a extins fiind imobilizate şi celulele plantelor superioare. De asemenea, s-au imobilizat protoplaste, organite celulare (cloroplaste, mitocondrii), precum şi organe sau organisme întregi (embrioni). Indiferent însă de celulele întrebuinţate,

* *Institutul de Cercetări Biologice, 3400 Cluj-Napoca, România*
** *Universitatea Babeş-Bolyai, Catedra de biologie vegetală, 3400 Cluj-Napoca, România*

procariote sau eucariote, se pune pregnant întrebarea: pe care le vom alege din ele și în ce scop? În acest sens va trebui să se țină seamă de o serie de criterii cum sunt: *a)* existența complexului sistem enzime — membrană celulară — perete celular — matrice; *b)* schimbul între substrat și celule (sau enzime); *c)* interacțiunea soluțiilor cu suprafața celulară; *d)* manifestarea reacțiilor chimice, care este de dorit să se petreacă în celulele intacte [23].

Metodele întrebuințate în prezent pentru imobilizare sunt subiective și de aceea este imposibilă o sugerare a unei metode universale. Cercetările recente se orientează spre găsirea unei matrice care permite o imobilizare facilă, sigură și o interacțiune bună cu substratul. După Novais [32], metodele de imobilizare cunoscute până în prezent pot fi clasificate după mai multe criterii, dar, în general, ele se împart în trei categorii mari:

1. imobilizarea prin legare de *a)* un suport solid (adsorbție fizică, legătură ionică și covalentă, legare de metal); *b)* legături intermoleculare sau intercelulare;
2. imobilizarea prin includere *a)* în geluri; *b)* în fibre; *c)* microîncapsulare; *d)* inserție în membrane;
3. peletizare, floculare, agregare.

Dacă imobilizarea enzimelor și a celulelor bacteriene are o vechime de cca. 30 de ani, imobilizarea celulelor plantelor superioare a fost efectuată pentru prima dată în anul 1979 de către Brodelius și colab. [7], prin includerea celulelor mai multor specii de plante ca și *Morinda citrifolia*, *Catharanthus roseus* și *Digitalis lanata* în gelul de alginat. De atunci tehnicile de imobilizare s-au diversificat, realizările obținute fiind semnalate de mai mulți autori [5, 10, 29, 32].

2. Scopul imobilizării celulelor plantelor superioare și aplicațiile acestei tehnici

Dezvoltarea proceselor biotehnologice pentru exploatarea impresionantului potențial biosintetic al celulelor plantelor superioare s-a focalizat în direcția propagării *in vitro* a țesuturilor și celulelor vegetale. Celulele cultivate sub formă de suspensii s-au bucurat de o atenție deosebită, datorită creșterii lor rapide și a posibilității de a fi cultivate în bioreactoare de tip industrial [18]. Totuși, cu anumite excepții, celulele vegetale cultivate *in vitro* sunt lipsite de capacitatea de a produce metaboliți secundari la același nivel cu cel al plantelor intacte [6, 12]. O explicație a acestui fenomen constă în aceea că suspensiile celulare realizate din plantele superioare sunt heterogene și nediferențiate, particularități ce conduc la o limitare a capacității lor de a avea o comunicare moleculară intercelulară [41]. În acest context s-a observat că la multe culturi celulare există o corelație inversă între intensitatea creșterii și cea a acumulării metaboliților secundari, și una directă între diferențierea celulară, declinul ratei de creștere și acumularea compușilor secundari [42]. De asemenea s-a constatat că la mai multe specii, cum ar

fi cele aparţinând familiei *Solanaceae*, agregarea sau „organizarea parţială" a celulelor în culturile de suspensii are ca efect mărirea sintezei metaboliţilor secundari [27]. În plus, există foarte multe dovezi care demonstrează că rata creşterii la culturile celulare cu un nivel ridicat de agregare este mică. În termeni mai simpli, fenomenul poate fi explicat prin faptul că, în condiţiile în care creşterea şi diviziunea rapidă sunt stimulate, precursorii vor fi utilizaţi pentru sinteza, de exemplu, a proteinelor, dar dacă creşterea este limitată, atunci aceiaşi precursori (aminoacizii) vor fi disponibili pentru sinteza metaboliţilor secundari, aşa cum sunt fenolii şi alcaloizii [29].

Între avantajele culturilor de celule vegetale imobilizate în scopul producerii de metaboliţi secundari mai pot fi incluse: 1. protecţia celulelor faţă de forţele de forfecare din lichid, care se nasc în vasul bioreactorului; 2. posibilitatea utilizării unor bioreactoare de volum mic în care biomasa imobilizată este menţinută într-o stare de creştere lentă şi într-un stadiu productiv pe o perioadă lungă; 3. separarea fazei de creştere de cea de producţie utilizând alternativ medii de creştere şi de producţie [8, 34]. În plus, mai mulţi autori au sugerat că mărirea stării de agregare şi organizare la celulele plantelor superioare imobilizate, în comparaţie cu suspensiile celulare, creează condiţii asemănătoare cu cele ale ţesuturilor plantelor [28, 36]. Contactul intercelular pe un timp îndelungat poate avea ca rezultat stabilizarea gradienţilor biochimici, stabilirea unei capacităţi mari pentru comunicarea intercelulară şi un grad rudimentar de diferenţiere cito- şi/sau biochimică [27, 28, 41].

Celulele vegetale imobilizate acumulează adesea cantităţi mai mari de metaboliţi secundari decât suspensiile celulare. De asemenea, există posibilitatea ca în urma imobilizării, celulele vegetale să elimine în mediu metaboliţi secundari care de obicei sunt stocaţi în vacuole. Celulele incluse în diferite matrice sunt utilizate şi în cazul reacţiilor de biotransformare, atunci când ele sunt capabile de a cataliza astfel de reacţii.

Desigur, există şi alte avantaje ale aplicării tehnicilor de imobilizare ale celulelor întregi. Astfel, cele mai multe studii s-au concentrat la început în testarea efectelor imobilizării asupra viabilităţii celulelor vegetale [29]. În plus, există posibilitatea de a stoca celule sau organite pe un timp îndelungat, facilitând în acest mod şi transportul lor [28]. De asemenea, tehnica poate fi şi este utilizată pentru prepararea semincţelor artificiale prin încapsularea embrionilor somatici în gel de alginat [33]. Această tehnică este valoroasă şi utilă în studierea fenomenului de recunoaştere celulară, a rolului agregării celulare în diferenţierea celulară şi în studiul răspunsului biochimic al celulelor vegetale la infecţii [29]. Avantajul culturilor celulare imobilizate mai constă şi în faptul că aceste sisteme sunt alcătuite din două părţi distincte, prima fiind reprezentată de celulele vegetale imobilizate într-o anumită matrice, iar a doua de mediul lichid nutritiv în care se găsesc aceste matrice. În cazul în care celulele excretă, în mod natural sau în urma unui tratament, metaboliţii secundari, ei vor fi recoltaţi cu uşurinţă din mediul de cultură, acesta fiind reutilizat în continuare.

3. Tehnicile imobilizării

Dintre toate tehnicile utilizate pentru imobilizarea culturilor celulare din plante superioare vom descrie mai pe larg doar câteva.

*3.1. Imobilizarea celulelor vegetale prin legare de un suport solid — **Adsorbția***

Mai mulți cercetători au imobilizat celulele vegetale prin adsorbția acestora pe diferite suporturi realizate din fibre de sticlă, polistiren sufonat, polistiren, tereftalat de polietenă, metale, ceramică. În special celulele de *Catharanthus roseus* au fost utilizate în astfel de experimente existând o amplă evidență pentru determinarea rolului tensiunii suprafeței celulelor și a suprafeței substratului imobilizator în procesul de adeziune [2, 16]. Această adeziune are loc în două etape distincte: *a)* interacțiunea inițială a celulelor plantelor superioare cu suprafața suportului mediată numai de forțe fizico-chimice; *b)* retenția intimă a celulelor plantelor pe substrat produsă prin secreția unor polizaharide și glicoproteine [3, 16, 17]. Cu toate că această tehnică reduce mult din costul operațiilor, aplicată la celulele de *Catharanthus roseus* nu a condus la rezultatele dorite, indiferent de suportul utilizat (tub de dializă, fibre de bumbac, fibre de sticlă, poliester) [15]. Fibrele de sticlă au fost utilizate și pentru imobilizarea celulelor de *Thalictrum rugosum*, cultivate în vase agitate sau în bioreactoare, dar berberina a fost produsă în cantități mai mici în comparație cu suspensiile celulare [14].

3.2. Includerea în geluri

a) Includerea în gel prin formarea unei rețele ionice

Metoda cel mai des utilizată pentru imobilizarea celulelor vegetale este includerea lor în gel. Cel mai comun gel este alginatul de calciu. Tehnica se bazează pe unirea a două molecule de acid alginic de către ioni bivalenți (Ca^{2+}, Ba^{2+} etc.) care se substituie ionului de Na, prezent în compoziția alginatului. Procesul este foarte simplu și constă în picurarea unui amestec de alginat de sodiu cu celule într-o soluție de $CaCl_2$, unde se formează structuri uniforme sferice, microporoase ce reține celulele. Alginatul se folosește cu preponderență, deoarece este ușor de manipulat și are o toxicitate foarte scăzută asupra celulelor vegetale. În plus, acest gel poate avea efecte benefice asupra sintezei metaboliților secundari. De la prima imobilizare a celulelor vegetale în alginat de calciu [7], numărul cercetătorilor care au aplicat această tehnică a crescut de la an la an. Astfel, A ș a d a și S h u l e r [4], imobilizând celulele de *Chataranthus roseus* în alginat de calciu, au obținut o mărire de 3 ori a cantității de ajmalicină extracelulară față de cea întâlnită în suspensiile celulare. De asemenea, K i m și Ch a n g [24] semnalează o sporire a producției de șiconină de 2,5 ori prin imobilizarea celulelor de *Lithospermum* în alginat de calciu. În cazul celulelor de *Thalictrum minus* [25] imobilizate în alginat de calciu, cu toate că producția (g/l/zi) de

berberină a fost mai mică decât a celulelor suspendate, ea s-a menținut o perioadă mult mai mare de timp. La celulele de *Berberis parvifolia* incluse în același tip de matrice, s-a constatat o sporire a cantității de alcaloizi protoberberinici, acumulați în celule sau excretați în mediu ($\mu g/cm^3/zi$) [11]. Ameliorarea producției prin imobilizarea celulelor în alginat de calciu a mai fost raportată și de V a n e k și colab. [38] în cazul biotransformării 2-(4-metoxi-benzil)-1-ciclohexanonei la glicozidul ei de către celulele de *Dioscorea deltoidea*.

b) Includerea în gel prin polimerizare

Un monomer sau o mixtură de monomeri este polimerizată în prezența suspensiei celulare, care va fi inclusă înăuntrul rețelei polimerului. Cel mai comun este poliacrilamida. Metoda se bazează pe polimerizarea radicalilor liberi ai acrilamidei într-o soluție apoasă. De cele mai multe ori, însă, poliacrilamida s-a dovedit a fi toxică pentru celule. Ea a fost utilizată pentru imobilizarea celulelar de *Catharanthus roseus* [5], dar cu rezultate slabe.

c) Includerea în gel prin precipitare

Gelurile se mai pot forma prin precipitarea unor polimeri naturali în urma modificării unuia sau mai multor parametri ai soluției, ca și: temperatură, salinitate, pH ori solvenți. În acest sens, s-au utilizat agarul, agaroza, gelatina și caraagenanul pentru imobilizarea celulelor de *Catharanthus roseus* [9], dar efectul lor pozitiv asupra sintezei de metaboliți secundari a fost mai slab decât cel al alginatului de calciu.

d) Includerea în structuri preformate

Pentru imobilizarea celulelor vegetale se mai pot utiliza și membrane fibroase (fabricate din fibre goale și permeabile) introduse în bioreactoare. Celulele împreună cu mediul lichid sunt trecute în aceste reactoare, fiind recirculate. Nutrienții cu greutate moleculară mică și produșii celulari trec ușor prin membrane, pe când celulele sunt reținute în ochiurile rețelei de fibre [37].

În alte cazuri, celulele sunt adăugate la structuri polimerice preformate așa cum este spuma de poliuretan. Celulele amestecate cu aceste materiale (tăiate sub formă de cuburi) migrează în interiorul rețelei și cresc în cavitățile poliuretanului. Creșterea este limitată prin restricție fizică. Metoda este simplă, dar are dezavantajul că sunt necesare cca. 10—15 zile pentru ca toate celulele să fie imobilizate. Tehnica a fost aplicată cu succes la celulele de *Dioscorea deltoidea* [21], unde s-a observat o mărire a sintezei de diosgenină. La aceeași plantă însă R o b e r t s o n și colab. [35] au constatat o descreștere a producției de diosgenină. Desigur, foarte multe experiențe sunt efectuate în vase conice agitate, dar ideal este ca matricele ce conțin celulele vegetale să fie introduse în bioreactoare, unde alimentarea cu nutrienți și recoltarea mediului se pot face mult mai ușor, automat, în mod continuu sau semicontinuu. Există foarte multe tipuri de bioreactoare în care pot fi cultivate ce-

lulele vegetale imobilizate în alginat de calciu [20], în poliuretan [29, 35], în fibre de sticlă [14, 15] sau în membrane realizate din fibre goale [37].

4. Efectele imobilizării asupra proprietăților celulelor vegetale

4.1. Metabolismul primar

Unii autori arată că în cazul sistemelor de cultură imobilizate există anumite limitări ale difuziei care pot conduce la reducerea eficienței acestor sisteme. Astfel, se modifică și proprietățile celulelor prin crearea unui micromediu în matricea imobilizată, care diferă de mediul înconjurător [32]. Probabil că acești autori se referă mai ales la celulele incluse în geluri. Totuși, s-au efectuat studii intense și în această direcție care scot în evidență faptul că atunci când se respectă anumite concentrații ale gelurilor, ele sunt perfect permeabile pentru nutrienți [29]. Așa este și cazul alginatului de calciu.

Desigur, proprietatea cea mai importantă pentru celulele imobilizate este viabilitatea. Ea poate fi apreciată prin determinarea: *a)* absorbției nutrenților și a oxigenului din mediu; *b)* a activității esterazice cu ajutorul diacetatului de fluoresceină [40]; *c)* a creșterii celulare. S-a constatat că cele mai toxice matrice pentru celulele vegetale sunt cele care necesită tratamente cu glutaraldehidă, așa cum este gelatina sau poliacrilamida [9].

Deși stabilitatea funcțională a celulelor imobilizate se poate modifica, totuși ea este mult mai mare decât a celor aflate în suspensii. Un fenomen care se întâlnește adesea în sistemele imobilizate este distrugerea matricelor (geluri de agar, agaroză, alginat) sau ieșirea lor din aceste matrice (de ex. poliuretan) datorită creșterii celulare. Dar și acest fenomen poate fi controlat prin alimentarea culturilor cu un mediu nutritiv limitator al creșterii (eliminarea din compoziția lui a fosfaților de eexmplu, sau a reglatorilor de creștere).

4.2. Metabolismul secundar

Cu toate că în multe cazuri s-au remarcat creșteri marcante în acumularea metaboliților secundari în celulele imobilizate, totuși în cazul acestor sisteme de culturi ni se pare a fi mult mai importantă eliminarea produșilor în exterior. În general, sunt extrem de puține specii, ale căror celule cultivate *in vitro* să excrete spontan compușii secundari în mediul de cultură. Cu toate acestea, există câteva excepții. Astfel, N a k a g a w a și colab. [30] au reușit să selecteze suspensii celulare de *Thalictrum minus* care există în mediu berberina, iar D e l i u (date nepublicate) a izolat câteva linii de *Berberis parvifolia* care elimină și ele, fără altă intervenție, alcaloizi protoberberinici. De asemenea, culturile celulare de *Coffea arabica* sunt capabile să producă alcaloizi ca și cofeina și teobromina, eliminând o mare parte din ei în mediu [26]. Desigur, astfel de culturi celulare se pretează foarte bine

pentru imobilizare, produşii excretaţi putând fi recoltaţi din mediu pe o perioadă îndelungată de timp. Se cunosc, de asemenea, puţine cazuri în care, în urma imobilizării, celulele să elimine produşi secundari. Totuşi, Lindsey şi Yeoman [29] arătau că prin includerea celulelor de *Capsicum fructescens* în alginat de calciu sau poliuretan se observă o eliminare puternică a capsaicinei. Acelaşi fenomen se constată şi la celulele de *Catharanthus roseus* imobilizate în alginat de calciu [4] care excretă ajmalicină.

Deoarece în sistemele de celule imobilizate se poate atinge o densitate celulară foarte mare, este posibil ca productivitatea, atât cea specifică (cantitatea de produs per unitate de greutate celulară per unitate de timp), cât şi cea volumetrică (cantitate de produs per unitate de volum — vas de cultură — per unitate de timp), să se mărească foarte mult în comparaţie cu celulele suspendate. Totuşi, acest fenomen nu se produce întotdeauna, observându-se chiar scăderi ale productivităţii. Aceasta demonstrează că, deşi de cele mai multe ori celulele îşi păstrează intactă activitatea enzimatică, ea poate fi redusă ca efect al naturii substratului utilizat pentru imobilizare.

În Tabelul 1, dăm doar câteva exemple ale influenţei pe care o poate avea natura matricei utilizate pentru imobilizarea celulelor, asupra sintezei metaboliţilor secundari.

Biotransformarea este o proprietate a celulelor plantelor superioare. Prin intermediul sistemului lor enzimatic, celulele unor specii cultivate *in vitro* pot să catalizeze transformarea anumitor compuşi (precursori) în alţii. Reacţiile de biotransformare realizate de culturile celulare vegetale sunt mai ales stereospecifice, implicând adiţia sau în-

Tabel 1

Efectul imobilizării culturilor celulare vegetale în diferite substrate asupra acumulării metaboliţilor secundari

Substratul imobilizant	Cultura celulară	Metabolitul secundar principial	Productivitatea specifică (faţă de culturile de suspensii)	Autori
Alginat	Morinda citrifolia	antrachinone	creşte	Brodelius şi Nilsson [9]
Alginat sau poliuretan	Capsicum fructescens	capsaicină	creşte	Lindsey şi Yeoman [29]
Fibre de sticlă	Thalictrum rugosam	berberină	descreşte	Facchini şi DiCosmo [14]
Alginat	Catharanthus roseus	ajmalicină	creşte	Brodelius şi Nilsson [9] Asada şi Shuler [4]
Alginat sau caraagenan	Catharanthus roseus	ajmalicină	creşte	Brodelius şi Nilsson [9]
Fibre de sticlă	Catharanthus roseus	ajmalicină, catarantină	descreşte	Facchini şi DiCosmo [13]
Poliuretan	Discorea deltoidea	diosgenină	creşte	Ishida [21]
Alginat	Nicotina tabacum	fenoli	creşte	Haigh şi Linden [20]

depărtarea unei singure grupări din compusul respectiv (hidroxilare, glicozilare, acetilare și metilare) [29]. Cel mai intens studiate au fost reacțiile de hidroxilare a digitoxinei sau a β-metildigitoxinei, reacții ce conduc la formarea digoxinei sau a β-metildigoxinei, prin intermediul culturilor celulare de *Digitalis lanata* imobilizate în diferite substrate [1] sau de *Daucus carota* [22]. Autorii respectivi au găsit, că în urma imobilizării, capacitatea de biotransformare a celulelor nu a crescut, dar ea a putut fi prelungită pentru o perioadă mare de timp. În acest context mai pot fi amintite și alte bioconversii notabile, realizate prin intermediul culturilor celulare imobilizate. Astfel, celulele de *Papaver somniferum* pot transforma codeionina în codeină [19], cele de *Coffea arabica* transformă teobromina în cofeină [26], iar celulele de *Nicotina tabacum* convertesc cetosterolii la hidroxiesteri [31].

5. Concluzii

În general, se poate spune că atât biotransformarea, cât și biosintezele complexe ce se produc în cazul celulelor plantelor superioare imobilizate, au avantaje distincte față de alte metode de cultivare a celulelor vegetale, deși această tehnică reclamă anumite tratamente în plus. Desigur, problema imobilizării este mai complexă, ea dând naștere, așa cum arăta Kennedy [23], la o serie de întrebări la care va trebui să se răspundă: *a*) care este efectul imobilizării asupra multiplelor procese ce au loc în celule?; *b*) determină imobilizarea schimbări caracteristice ale compoziției suprafeței celulelor sau ale conținutului lor enzimatic?; *c*) se poate produce fenomenul de „rejecție" la contactul dintre celule și matrice sau se vor genera produși suplimentari cu sau fără toxicitate?

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LETTUCE, LAMBSQUARTERS AND COUNTRY MALLOW CALLUS CULTURE BIOASSAYS IN THE STUDY OF ALLELOPATHY

ARMERIA VICOL* and CRISTINA DOBROTĂ**

SUMMARY. — Aqueous rye (*Secale cereale* L.) extract prepared from fresh plant material inhibited the growth of lettuce (*Lactuca sativa* L.) and lambsquarters (*Chenopodium album* L.) calluses. Lettuce radicle growth was affected by rye extract in a similar way, which confirmed the analogy between white cell cultures and plant roots. Callus cultures of country mallow (*Malva parviflora* L.) were obtained for the first time and their growth was found to be inhibited by the aqueous extract of wild mustard (*Brassica kaber* (D.C.) Wheeler). The potential role of cell culture bioassays in the study of allelopathy against species with hard-to-break seed dormancy is proposed.

There are various control methods to reduce weed problems [10], but many are either labour intensive, or imply the use of herbicides, which are potential hazards for man. Emphasis is growing presently for the use of the biochemical interaction between plants — allelopathy — for weed management. Considerable attention has been given by now to the allelopathic interactions between different crop plants or between crops and weeds. The possibility also exists that the inhibitory effects of phytotoxins produced by some weed species on others be used for weed management, as a weed may inhibit the invasion of other weeds through a combination of allelopathic and competitive interference [11].

Several bioassays have been designed and used in the study of allelopathy, such as seed germination [9, 12, 13, 16, 18], radicle elongation [1, 8, 15], and, rarely, coleoptile growth [2] and tissue cultures [5, 7]. The first two categories of bioassays can be difficult to use in the case of plants with a hard-to-break seed dormancy, which is often the case with weed species. Cell cultures may then represent a useful alternative. G r e s s e l [4] presented a possible analogy between plants and cell cultures of different colours and ages. It may be thus possible that white, fast-growing calluses replace seedlings in allelopathy bioassays that monitor root growth, and green calluses represent suitable bioassays when the allelochemical is thought to inhibit photosynthesis.

This study tested the analogy between white calluses and roots, using lettuce (*Lactuca sativa* L.) as a widely used test species in allelopathy experiments. Callus cultures of two weeds, lambsquarters (*Chenopodium album* L.) and country mallow (*Malva parviflora* L.) with low germination percentages due to seed dormancy were then used to study

* *Biological Research Institute, 3400 Cluj, Romania*
** *Babeş-Bolyai University, Department of Plant Physiology, 3400 Cluj, Romania*

the potential allelopathic inhibition by rye (*Secale cereale* L.) and wild mustard (*Brassica kaber* (D.C.) Wheeler), respectively.

Materials and methods. Lettuce callus cultures were established from 7-day-old, aseptically germinated seedlings. The culture medium used for callus initiation contained: macronutrients according to Murashige and Skoog [14] 100 ml/l, micronutrients according to Heller [6], 1 ml/l, FeEDTA 5 ml/l, *myo*-inositol 100 mg/l, pyridoxine. HCl, thiamine. HCl, nicotinic acid, α-naphthylacetic acid and benzyladenine, 1 mg/l each, sucrose 20 g/l, and Bacto Difco Agar 7 g/l. The pH was adjusted to 5.8 before autoclaving (121°C, 20 min.). Four ml of medium were prepared per vital (scintillation vials of 20 ml). White calluses formed mainly from hypocotyls were maintained by transfer every four weeks to a fresh medium with the same composition.

Callus cultures of lambsquarters derived from stem internode segments (approximately 1 cm in length) excised from field-grown plants. The cuttings were sterilized with 2.62 % sodium hypochlorite (50% commercial bleach) for 15 min., and rinsed six times with sterile, deionized water. The medium used for callus initiation and maintenance was the same as for lettuce, and transfer to fresh medium was performed at the same 4-week interval.

In country mallow, a range of explant types was screened for their ability to from callus on three different media (Table 1). Explants were collected from mature plants grown in the field and sterilized with 2.62% sodium hypochlorite for 7 to 15 min. according to the explant type. The success in callus induction depended mostly on the type of expant used and not on the medium. All subsequent experiments were done with calluses derived from stem internodes, on the same medium as the one used for lettuce and lambsquarters.

Callus cultures were maintained by repeated transfer every four weeks. All calluses were incubated in the light (2000 lux, 16 hrs/day), at 25±2°C.

All experiments with lettuce and lambsquarters used a 20% rye extract, obtained by soaking 20 g of fresh, whole, 30-day-old rye plants in 100 ml of deionized water, for 12 hours, followed by filtration through Whatman No. 1 filter paper. The extract had an osmotic concentration of 33 mOsm and pH 6.2.

Table 1

Media used for callus induction in country mallow

Medium composition	I	II	III
Macronutrients MS (ml/l)	100	—	100
Micronutrients MS (ml/l)	—	—	1
Macronutrients B5* (ml/l)	—	100	—
Micronutrients B5 (ml/l)	—	1	—
Micronutrients Heller (ml/l)	1	—	—
FeEDTA (ml/l)	5	5	5
myo-Inositol (mg/l)	100	100	100
Pyridoxine·HCl (ml/l)	1	1	0.2
Thiamine·HCl (mg/l)	1	2	0.4
Nicotinic acid (mg/l)	1	1	0.2
α-Naphthylacetic acid (mg/l)	1	—	—
2,4-D (mg/l)	—	5	1
Benzyladenine (mg/l)	1	0.1	2.5
Casein hydrolysate (mg/l)	—	2	—
Sucrose (g/l)	20	20	20
Agar (g/l)	7	6.5	6
pH	5.8	5.5	5.6

* The mixture of macronutrients B5, as well as micronutrients B5 was designed by Gamborg *et al.* [3].

Country mallow experiments were done with a 20% fresh wild mustard extract prepared from whole 30-day-old plants in the same way as the rye extract. The osmotic concentration was 33 mOsm and the pH was 6.5.

Lettuce seeds were sterilized with 2.62% sodium hypochlorite for 10 min., then rinsed six times with sterile, deionized water and placed on agar media, in 20 ml scintillation vials with screw caps. The basic medium we used was Murashige and Skoog [14], without growth regulators and solidified with 0.7% agar. Two treatments were used: 1. the water needed for medium preparation was replaced by the extract; the medium was adjusted to pH 5.8 and was autoclaved for 20 min. (121°C); 2. the medium contained water and extract in a proportion of 1:1; the extract was added after autoclaving, by filter-sterilization using Nalgene disposable filters with low-protein binding membranes. Twenty vials were prepared per treatment, with 4 ml of medium per vial. The seeds were incubated in the dark, at 25±2°C, for 10 days.

The treatments for lettuce and lambsquarters calluses were represented by the basic medium used for their induction and maintenance, modified by the addition of rye extract in a way similar to that described for the lettuce radicle growth bioassay. A medium containing water and wild mustard extract (1:1, w/w) was prepared for country mallow calluses. The extract was added after filter-sterilization with Nalgene filters. Twenty vials were prepared per treatment (4 ml medium/vial).

Preweighed pieces of callus tissue from all three species (three weeks from the last subculture) were allowed to grow under treatment conditions (2000 lux, 16 hrs/day, 25±2°C) for a month before reweighing. Data are expressed as percent increase in gram fresh weight, compared to the control.

Results and discussion. Lettuce radicle growth was significantly reduced by both the 20% autoclaved rye extract and the 10% non-autoclaved one, but more by the first extract than by the second. The same pattern of inhibition was shown by the lettuce callus bioassay. This finding supports Gressel's [4] analogy between white callus cultures and the root (and shoot) meristem, and suggests that such cell cultures respond to allelochemicals in a way that is similar to plant roots. If this is true, then cell cultures could replace the radicle growth bioassay in species with a hard-to-break seed dormancy, more common within weeds than crops. We have chosen lambsquarters and country mallow as two such species. Lambsquarters callus growth was inhibited by both concentrations of rye extract, but to a lesser extent than was the lettuce callus (Table 2). This suggested that rye had allelopathic

Table 2

The effect of rye extract on lettuce and lambsquarters callus and on lettuce radicle growth

Bioassay	Treatment	Radicle length (% of the control)	Increase in fresh weight (% compared to the control)
Lettuce radicle	20% rye extract	55.73a*	—
	10% rye extract	74.72b	—
Lettuce callus	20% rye extract	—	56.75a
	10% rye extract	—	64.19b
Lambsquarters callus	20% rye extract	—	63.69a
	10% rye extract	—	75.21b

* Numbers followed by the same letter in a column are not significantly different at p = 005.

potential against this weed. Field data [17] support this possibility, indicating that autumn planted, spring killed rye reduced the biomass of several weeds, among which lambsquarters.

Country mallow has not been studied in tissue culture by now. The possibilities of inducing callus from different explants are described in Table 3. Only calluses produced by stem internodes on medium I were used in the allelopathy experiments.

Country mallow callus growth was also inhibited by the 10% fresh, wild mustard extract .Table 4). The allelopathic potential demonstrated

Table 3

Callus induction and morphology from different explants of country mallow

Explant type	Culture medium I			II			III		
	No. expl.	Calluses (%)	Callus type*	No. expl.	Calluses (%)	Callus type	No. expl.	Calluses (%)	Callus type
Mature leaf blade	18	44	2	13	46	2	26	3.9	2
Young leaf blade	17	20	2	20	30	3	27	7.4	3
Fruit with peduncle	14	14	2	11	27	4	20	0	1
Petiole	12	33	2	10	60	3—4	20	60	3
Stem node	16	87	5	31	81	3—5	14	86	3
Stem internode	24	92	5	30	83	3—5	15	93	3
Flower bud	20	10	4	18	22	4	20	0	1

* 1 — No callus formed. 2 — A little callus formed at the edge of the explant. 3 — Beige, friable, fast-growing callus. 4 — Wihite, nodular callus. 5 — Cream callus with green nodules, friable, medium-growing.

Table 4

The effect of wild mustard extract on country mallow callus growth

Treatment	Increase in fresh weight (%)
Control	190.79
Wild mustard extract	116.82

by the extract on the callus of this species suggests that allelopathy may be a mechanism by which wild mustard inhibits country mallow under natural conditions.

Conclusions. 1. Lettuce and lambsquarters callus growth was inhibited by aqueous rye extracts. Since the growth of lettuce radicles was also affected by rye extracts, it is possible that an analogy be drawn between white calluses and plant roots. Therefore, lambsquarters radicle growth may also be inhibited by rye — a possibility with valuable applications in the management of this weed.

2. Callus cultures of another weed, country mallow, were inhibited by aqueous wild mustard extract. The practical importance of this

finding is supported by field observations, which show that wild mustard actually hampers country mallow growth.

3. Both lambsquarters and country mallow have seeds that cannot be easily used in allelopathy studies, because their dormancy is difficult to break. Callus cultures may represent a useful alternative for studying biochemical interactions with such species.

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DICLOFENAC AND ASPIRIN INTERFERE WITH GLUCOSE METABOLISM AND AFFECT ULTRASTRUCTURE OF THE PERFUSED LIVER

IOAN PETRESCU*, CORNELIU TARBA*, CONSTNTIN CRĂCIUN and VERONICA CRĂCIUN****

SUMMARY. — The actions of the non-steroidal anti-inflammatory drugs (NSAIDs) diclofenac and aspirin on gluconeogenetic and glycolytic metabolism of the perfused rat liver were investigated. Diclofenac or aspirin was infused to livers isolated both from 48-hr fasted rats and well-fed rats. The former were perfused with Krebs-Henseleit bicarbonate buffer containing L-lactate (2 mM) and pyruvate (0.1 mM), allowing the measurement of glucose synthesis (gluconeogenesis). Both diclofenac (0.01—0.1 mM) and aspirin (1—10 mM) have inhibitory effects on glucose formation. A marked inhibitory effect was observed at 0.1 mM for diclofenac and 10 mM for aspirin. For both drugs, the effects were reversible. The livers from well-fed rats were perfused with substrate-free buffer, allowing the measurement of glycogen metabolism. The rates of glucose release and of lactate and pyruvate production provide a good estimation of glycogenolysis and glycolysis from endogenous glycogen. Both diclofenac (0.1 mM) and aspirin (5 mM) increased glycogenolysis and glycolysis (GGL/GL). Oxygen consumption was continuously monitored with a Clark oxygen electrode. In general terms, the effects of diclofenac and aspirin on gluconeogenesis (GNG) and glycogenolysis/glycolysis seem to be the primary consequence of their uncoupling action, a conclusion which is supported mainly by the opposite effects on gluconeogenesis (*i.e.*, inhibition) and glycogenolysis/glycolysis (*i.e.*, stimulation), along with an increased oxygen consumption. The ultrastructural study of both rat and guinea-pig perfused livers also supports the idea of the uncoupling effect of diclofenac, as suggested by the strong decrease in glycogen quantity and the overwhelming presence of swollen mitochondria, displaying rarefied matrix and cristae.

For over 20 years, since Vane [27] has proposed a plausible hypothesis on the mechanism of action of aspirin and related compounds, it has been generally accepted that non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting the biosynthesis of certain prostaglandins. Although several discrepancies with such a hypothesis have been reported over this period of time, it was not until recently that the prostaglandin hypothesis has been challenged. Based on both literature data and their own results, Abramson and Weismann [2] advanced a new hypothesis on the mechanism of action of NSAIDs. In short, their hypothesis is based on the ability of such drugs to interact with the biological membranes and to disrupt certain membrane-dependent processes, among which signal transduction at the level of plasmalemma is

* *Babeş-Bolyai University, Department of Animal Physiology and* ***Laboratory of Electron Microscopy, 3400 Cluj-Napoca, Romania*

considered of primary importance. Indeed, aspirin and other NSAIDs are capable of interfering with the stimulus-response coupling in the neutrophils, the most abundant cells of acute inflammation, preventing the aggregation of such cells and the consecutive release of various inflammatory factors [2, 29]. It is not our intention to present evidence against or in favour of either hypothesis but rather to point to the complexity of the problem. While inhibition of prostaglandin biosynthesis seems to be involved in producing the analgesic and antipyretic effects of NSAIDs, higher concentrations, which are needed for the anti-inflammatory action, inhibit a series of nonprostaglandin-dependent reactions (see [1, 3, 4, 20, 21]). Moreover, Baggot *et al.* [3] consider the inhibition of folate-dependent enzymes by these drugs as crucial for the mechanism of action of NSAIDs.

Very important for our work, however, is the observation that NSAIDs interfere with the energy metabolism in both isolated mitochondria [15] and hepatocytes [5] and also in the perfused rat liver [14]. Due to the great importance of energy metabolism, to the fact that energy transduction in mitochondria depends on the integrity of the inner mitochondrial membrane, and considering our own results regarding the effects of one analgesic and several local anesthetics on mitochondria [23—25], we decided to undertake a deeper and broader study of the effects of two important NSAIDs (aspirin and diclofenac) on the perfused liver and on the isolated hepatic mitochondria of two species (rat and guinea pig).

The present article describes the effects of diclofenac and aspirin on glucose metabolism in the perfused liver, while our accompanying paper [26] is dedicated to isolated rat and guinea pig liver mitochondria. The liver perfusion technique was selected because it is well known that, due to the absence of any biochemical interference with other organs, it represents one of the best biochemical and physiological models. For more confidence in our results, we also decided to complete the biochemical (functional) assays with electron microscopy, capable of providing a good structure-function correlation.

Material and methods. *Chemicals.* Biochemica Test-Combination kits for glucose (GOD-Perid), lactate and pyruvate and all enzymes and coenzymes used for assays were from Boehringer-Mannheim GmbH. Pyruvate was from Merck (Darmstadt) and lactate from Riedel-de-Haën (Hanover). Diclofenac (sodium *o*-[2,6-dichlorophenyl]-aminophenyl acetate) and aspirin (acetylsalicylic acid) were from Romanian Drug Enterprises (Bucharest). All other chemicals were of highest purity commercially available.

Animals. Male albino rats (Wistar strain, 200—250 g) were used in our studies. Well-fed rats were employed as liver donors for glycogenolysis and glycolysis studies, while rats with free access to water but fasted for 48 hrs were used for GNG studies. The animals were anesthesized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). In addition, male guinea pigs (a mixed strain, 250—300 g) were also used for studies of electron microscopy.

Liver perfusion. The liver was perfused *in situ*, after removing the viscera, as described in [9] and [13]. The liver was cannulated via the hepatic portal vein (for influx) and the supradiaphragmatic portion of the inferior vena cava (for efflux). A non-recirculating (*i.e.*, through-flow) hemoglobin-free perfusion was used. The perfusion medium, Krebs-Henseleit bicarbonate buffer, pH 7.3—7.4, was

pumped into the liver with the aid of a peristaltic pump, at a rate of 32.5—33.0 ml/min. The flow rate was constant for the same liver during the entire period of perfusion. The perfusion medium was continuously gassed with O_2:CO_2 (19:1) and oxygenated by a rotatory disc oxygenator. The glucose precursors (*i.e.*, lactate and pyruvate) and the drugs were dissolved in the perfusion medium and pumped into an in-line infusion chamber with the aid of an infusion pump, so as to enter the liver at a constant rate (0.2—0.4 ml/min) and to give the desired final concentration in the perfusion medium. When necessary, the compounds to be presented to the liver were brought to pH 7.4 with either NaOH or HCl. Oxygen consumption by the liver was determined using an in-line oxygen electrode attached to a potentiometric recorder. A value of 2.07 µatoms of oxygen per ml perfusion medium was used as a basis for calculating oxygen consumption.

Drug concentrations administered in the perfusion were in the usual range employed in such studies. Thus, diclofenac was varied from 0.01 to 0.1 mM, whereas aspirin from 1 to 10 mM. These figures parallel the normal clinical doses used in inflammation treatments, *i.e.* 150 mg/day for diclofenac [19] or 5—8 g/day for aspirin (see Table 1 in Baggot *et al.* [3]). Even though the concentrations used by us may be somewhat higher than those attained in *in vivo* treatments, they cannot be much higher, since, according to the same Table 1 in [3], peak plasma concentrations of aspirin in patients treated for inflammatory diseases is around 2 mM and the tissue concentration cca. 12 mM.

Sampling and metabolite assays. Effluent samples were collected at 3-min intervals and assayed spectrophotometrically for glucose, lactate and pyruvate, immediately after the perfusion end, using the appropriate Biochemica-Combination kits, depending on the experiment.

Electron microscopy. For comparing the ultrastructural aspects of liver before and after the perfusion with diclofenac, the following procedure was used. After 10 min of perfusion with buffer and 5.5 mM glucose, one of the hepatic lobes was ligated and a small fragment was taken from it and prepared for electron microscopy. This represented the so-called perfused control. The rest of the liver was further perfused with diclofenac for 30 min, after which a fragment from a different lobe was taken for electron microscopy. In parallel, we also prepared an unperfused control, obtained from an animal which was not perfused at all. The fragments were fixed with a 2.7% glutaraldehyde solution in 0.15 mM phosphate buffer, pH 7.2, at 4°C, for 2 hrs. The washing was performed in four successive baths, with the same phosphate buffer, for 4 hrs, after which the tissue was postfixed with a 1% osmic acid solution in 0.1 M phosphate buffer, pH 7.2, for 1 hr. The samples were further dehydrated in acetone solutions of increasing concentration, infiltrated and embedded into a polyester resin (Vestopal W). The sectioning was performed with an LKB-III ultramicrotome, the sections contrasted with uranyl acetate and lead citrate and examined in a TESLA-BS-500 electron microscope. The method and working technique were those currently used in the electron-microscopic investigation (see, for ex., [28]).

Results. *Effects of diclofenac and aspirin on hepatic gluconeogenesis.* For complete depletion of the hepatic glycogen reserve, 48-hr fasted rats were used in the study of GNG. Lactate (2 mM) and pyruvate (0.1 mM) (final concentrations in the perfusion medium) were employed as glucose precursors (substrates).

As can be seen in Fig. 1, the first 15 min of perfusion with Krebs-Henseleit bicarbonate medium alone are characterized by a very low content of glucose in the effluent. The addition of lactate and pyruvate in the perfusion medium induces a very rapid increase of glucose synthesis, which remains constant over the entire period of substrate administration. The interruption of the substrate administration results in a drastic decrease of glucose formation. As can also be seen from

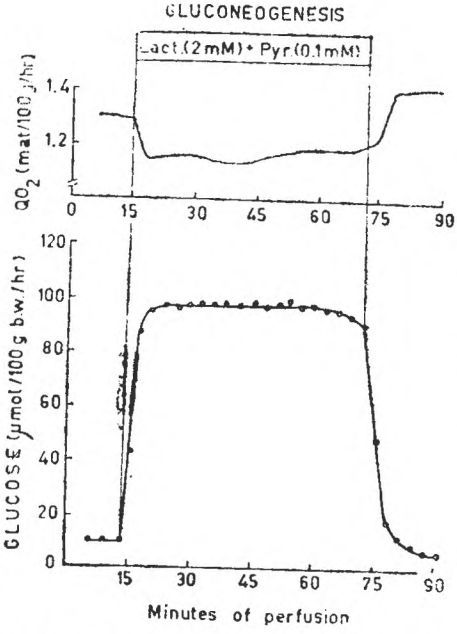

Fig. 1. *Evolution of glucose synthesis and oxygen content in the effluent of the perfused control rat liver.* The conditions are as described in „Materials and methods". For more explanations, see the text.

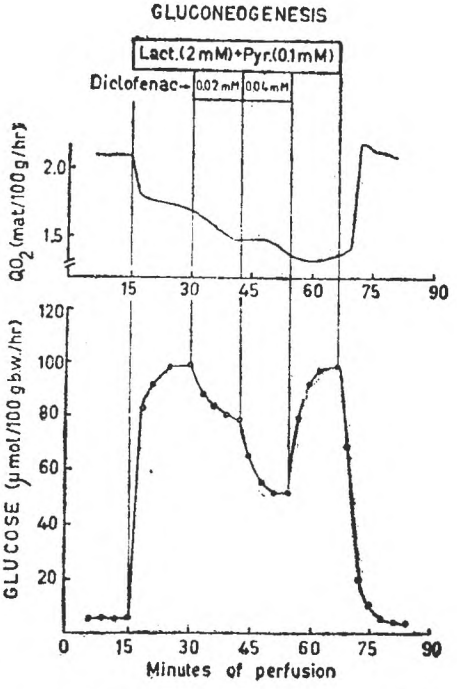

Fig. 2. *Effect of successive additions of low concentrations of diclofenac on glucose synthesis and oxygen evolution in the perfused rat liver.*

the figure, the oxygen content in the effluent during substrate administration decreases, indicating an increase of oxygen consumption by the hepatic tissue, GNG being a highly endergonic process.

Diclofenac administration (0.02—0.1 mM) determines a decrease of GNG (Figs. 2—3). The inhibition of glucose synthesis is proportional to diclofenac concentration and lasts as long as the drug is present in the perfusion medium. The interruption of diclofenac administration restores the normal glucose synthesis. It is worth noting that oxygen consumption also increases in the presence of diclofenac (see especially Fig. 2). However, at 0.1 mM drug (Fig. 3), where GNG is 80% inhibited, after an initial increase, the trend of oxygen consumption reverses.

As in the case of diclofenac, the presence of aspirin (1—10 mM) in the perfusion medium induces the inhibition of GNG and an increase of oxygen consumption (Figs. 4—6). Similarly, the inhibition is proportional to the drug concentration and it is reversible (Figs. 4—5). While 10 mM aspirin inhibits completely GNG, an „overshoot" of glucose production can be seen after suppressing the administration of this high concentration of aspirin (Fig. 6). However, such metabolic overshoots are

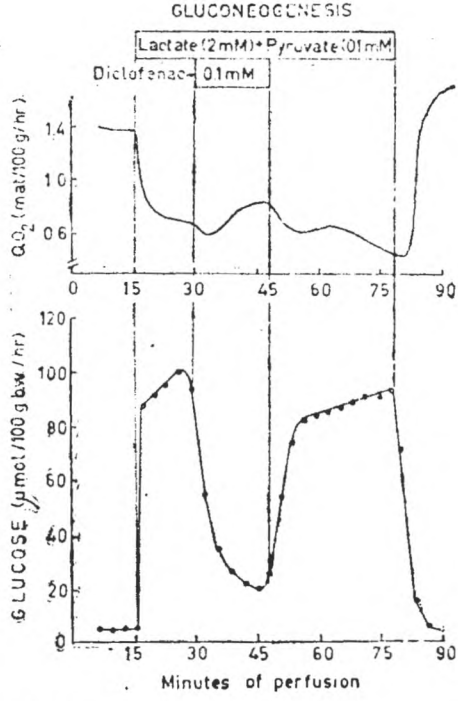

Fig. 3. *Strong effect of a high concentration of diclofenac* (0.1 mM) *on glucose synthesis and oxygen evolution in the perfused rat liver.*

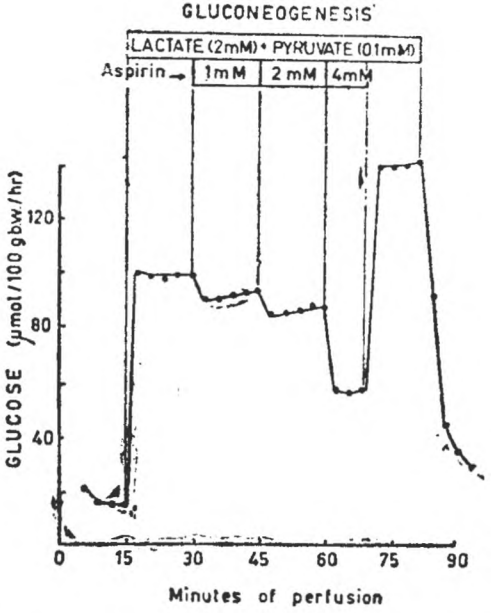

Fig. 4. *Effect of increasing concentrations of aspirin on gluconeogenesis in the perfused rat liver.*

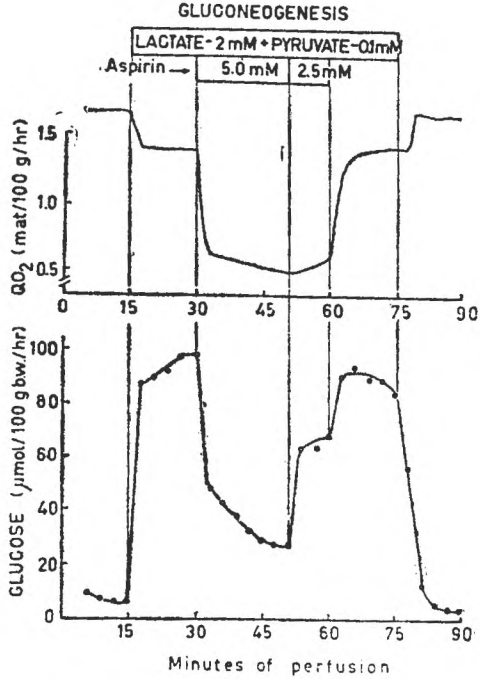

Fig. 5. *Effect of decreasing concentrations of aspirin on glucose synthesis and oxygen evolution in the perfused rat liver.*

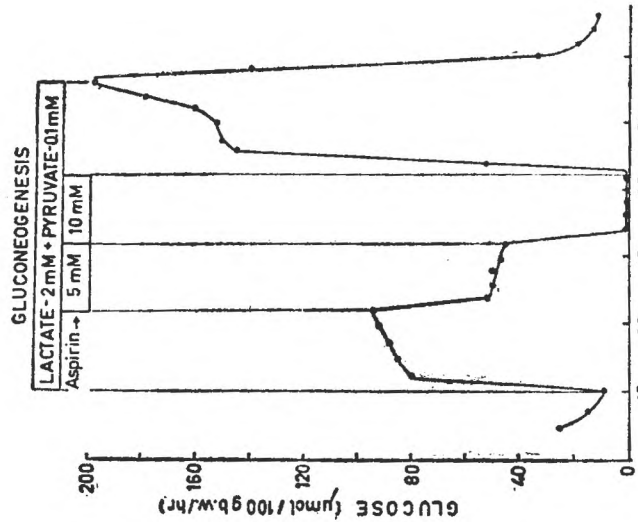

Fig. 6. *Strong inhibitory effect of high concentrations of aspirin on gluconeogenesis in the perfused rat liver.*

rather common events and we do not intend to advance here a disputable explanation, especially since it does not interfere with our further presentation.

Effcts of diclofenac and aspirin on hepatic glycogenolysis and glycolysis. The effects on GGL and GL were studied on livers obtained from well-fed rats perfused with medium without substrate. The intensity of glycogenolysis was estimated by measuring glucose liberation from endogenous glycogen, whereas the intensity of glycolysis was estimated through the lactate and pyruvate originating from glucose catabolism. Oxygen concentration in the effluent was also monitored.

As can be seen from Fig. 7, the administration of diclofenac (0.1 mM) in the perfusion medium determines an increase of both GGL and GL. A slight increase of oxygen consumption can also be observed. The effects are reversible for all the parameters tested.

Aspirin (5 mM) also produces an increase of both GGL and GL (Fig. 8). The visible decrease of oxygen concentration in the effluent indicates a strong increase of oxygen consumption by the liver tissue during aspirin administration. The effects are, again, reversible.

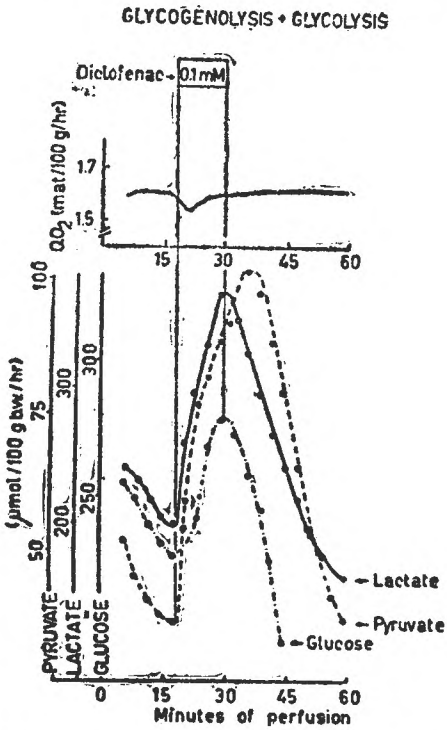

Fig. 7. *Effect of a high concentration of diclofenac (0.1 mM) on glycogenolysis, glycolysis and oxygen evolution in the perfused rat liver.*

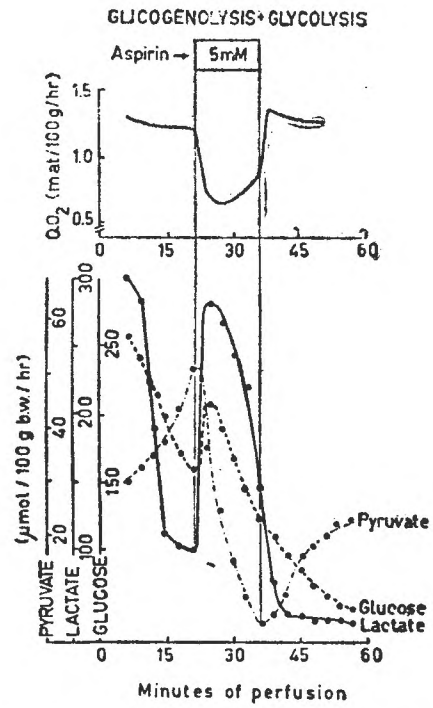

Fig. 8. *Effect of a high concentration of aspirin (5 mM) on glycogenolysis, glycolysis and oxygen evolution in the perfused rat liver.*

Ultrastructure of rat and guinea pig livers perfused with diclofenac. To follow the effects of diclofenac on the *in situ* hepatocyte ultrastructure, we selected the concentration which had a maximal effect in the biochemical experiments, *i.e.* 0.1 mM.

Rat liver. Fig. 9a presents aspects of rat hepatic tissue perfused with medium plus glucose (5.5 mM) for 10 min. As compared to the unperfused tissue (not presented here), the hepatocytes show minor modifications, such as:

— a slight tendency of mitochondria to swell and a rarefaction of their matrix, especially in the centrolobular zone;

— a slight retention of lipid droplets in the hepatocytes.

The other organelles and cell components have characteristics similar to those present in the unperfused liver.

Practically, the same characteristics are seen if glucose is not present in the perfusate (Fig. 9b).

Addition of 0.1 mM diclofenac and further perfusion for 30 min (Fig. 9c) results in the following ultrastructural alterations:

— swelling of mitochondria, accompanied by an extensive rarefaction of mitochondrial matrix and cristae;

— loss of the spherical form of the nuclei, their contour becoming irregular;

— increase in the amount of smooth endoplasmic reticulum, a usual reaction to hepatotoxic drugs;

— decrease in the number of glycogen microparticles;

— increase of the number of lysosomal formations;

— slight dilation of the biliary canaliculi.

Guinea pig liver. Unlike the rat hepatocyte, the normal guinea pig hepatocyte has the following ultrastructural characteristics (Fig. 10a):

— the nucleus usually possesses 2—3 nucleoli with a reticulate ultrastructure;

— the number of mitochondria per unit volume is smaller;

— the number of glycogen microparticles is larger, often disposed in aggregates;

— the number of lipid droplets is smaller.

The guinea pig liver perfused for 10 min with medium plus glucose (Fig. 10b) does not show notable differences as compared to the unperfused liver.

Addition of 0.1 mM diclofenac and further perfusion for 30 min (Fig. 10c) produces alterations similar to those observed in the rat liver:

— swelling of mitochondria (until they become spherical) and rarefaction of their matrix and cristae;

— drastic decrease in glycogen quantity, with the disappearance of the glycogen aggregates;

— loss of the spherical form of the nuclei and increase in the quantity of nuclear heterochromatin, with repercursions on the synthesis of structural proteins;

— presence of mainly inactive Kupffer cells, suggesting the alteration of their defense system.

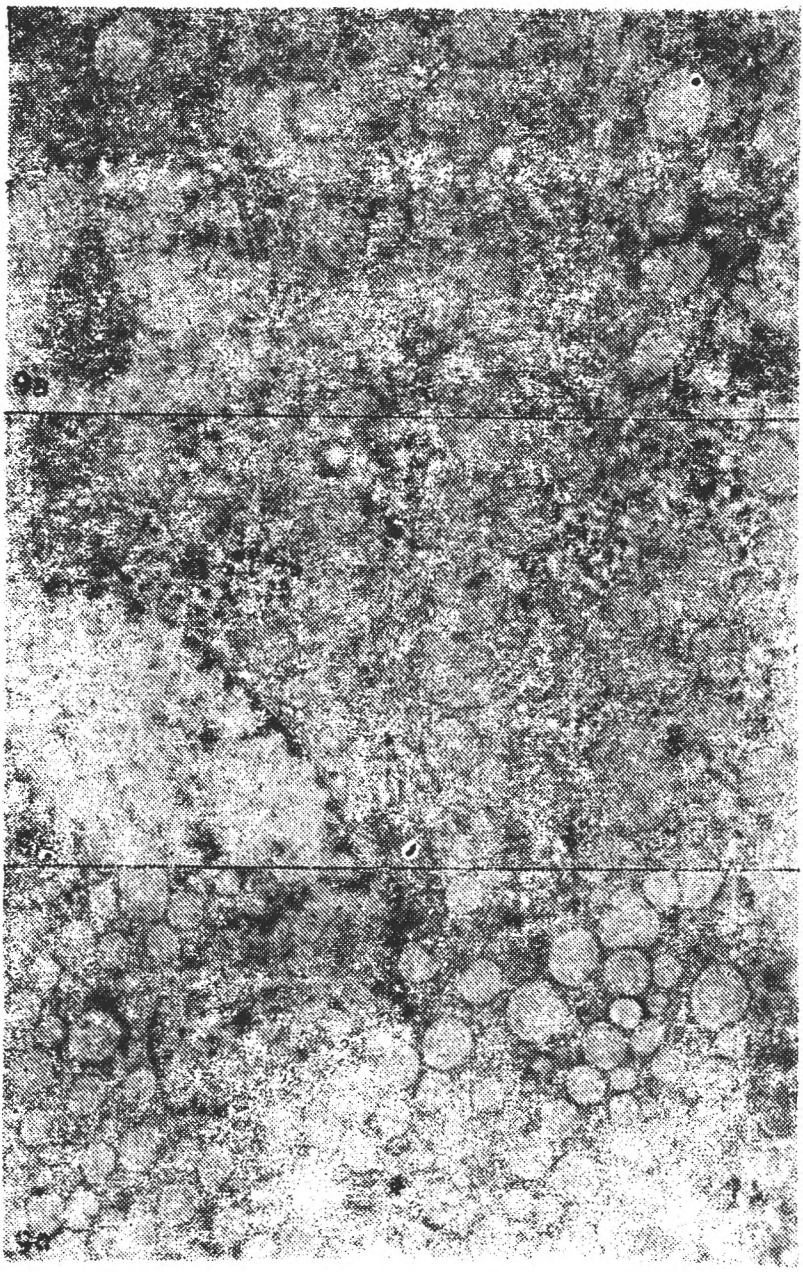

Fig. 9a. *Ultrastructural aspects of the rat liver perfused for 10 min with Krebs-Henseleit medium plus 5.5 mM glucose (perfused control)* (X 8.220).
9b — *As in 9a, but without glucose* (X 15,750). 9c — *Effects of a 30—min perfusion with diclofenac (0.1 mM) on the ultrastructure of rat liver* (X 5,480). The rest of the conditions are as described in 9a. For more explanations, see the text.

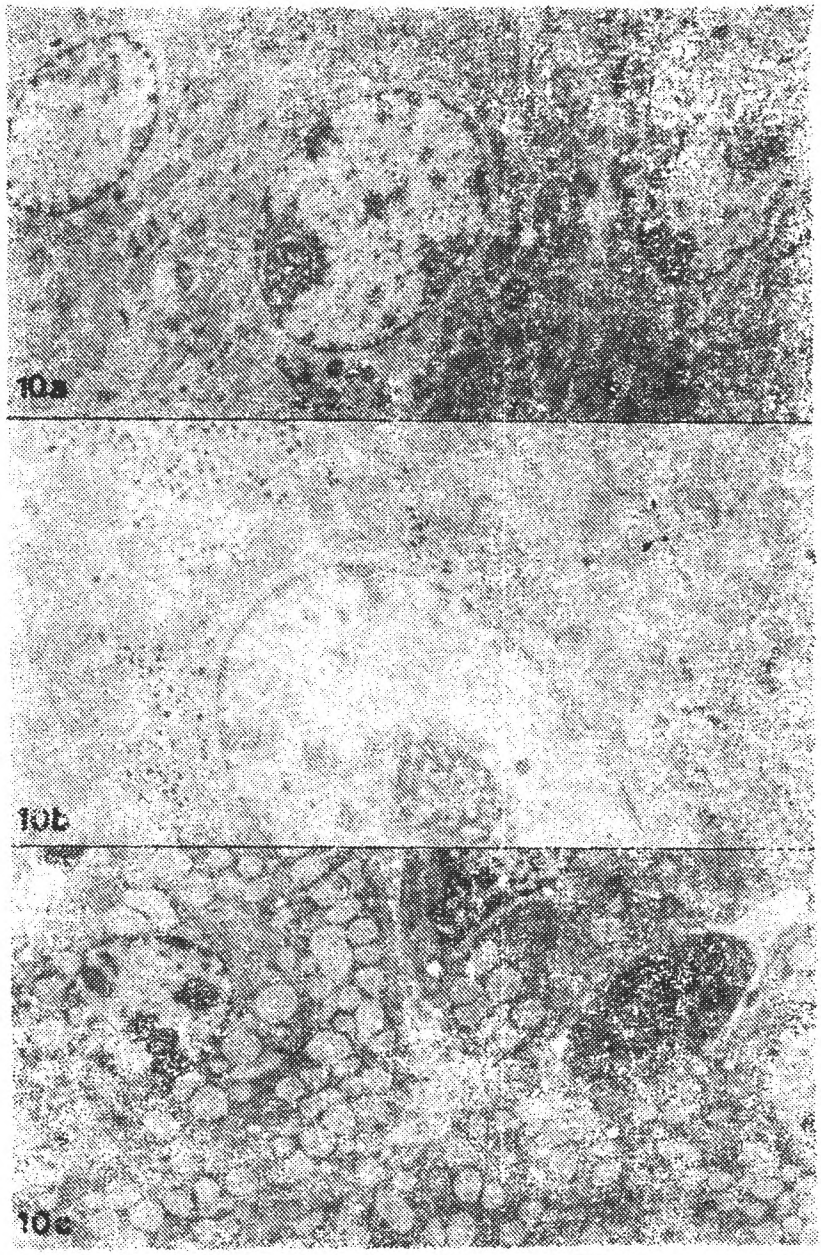

Fig. 10 a. *Ultrastructure of unperfused guinea pig liver* (X 4, 650). 10b — *Guinea pig liver perfused for 10 min with Krebs-Henseleit medium plus 5.5 mM glucose* (X 8,220). 10c — *Effects of a 30—min perfusion with diclofenac (0.1 mM)* (X 3,850). The rest of the conditions are as described in 10b. See also the text.

Discussion. From the results presented above, the effects of diclofenac and aspirin on carbohydrate metabolism can be summarized in terms of a stimulation of glycogenolysis/glycosis and an inhibition of gluconeogenesis. As it is well known, glycolysis is the only source of energy in the eukaryotic cell under anaerobic conditions or when oxidative phosphorylation is uncoupled. By releasing the pressure exerted on the electron flow, uncoupling stimulates the respiration in a futile cycle. For thermodynamic reasons, under these conditions, glycogenolysis and glycolysis are also expected to be stimulated and produce the necessary ATP for the cell. Thus, the stimulating effects of the two drugs on GGL/GL could be the result of an uncoupling process. In fact, our accompanying paper [26], dealing with isolated mitochondria, provides direct evidence for the uncoupling phenomenon. Even though, in the present work, we did not measure the content of adenine nucleotides, we have done this in the case of Boicil (an alcoholic extract from *Helleborus* sp. used in the Romanian clinics as an analgesic) and found that after the perfusion with Boicil the quantity of adenine nucleotides (especially that of ATP) decreased appreciably (P e t r e s c u, I., unpublished data). We do have, however, even in the present paper, additional evidence that the two drugs promote the uncoupling of mitochondria. The electron-microscopic results, showing swelling of mitochondria and rarefaction of their matrix and cristae, are indices of an uncoupling phenomenon, as shown by us for similar uncoupling agents [23—25]. The other ultrastructural aspects, such as the decrease in the quantity of glycogen and lipids as well as the changes in the morphology of the nucleus and other organelles represent either a direct or an indirect confirmation of the biochemical results. Such alterations of the mitochondrial structure are generally considered by M u n n [17] as certifying the impairment of the reactions of oxidative phosphorylation and can be often observed in the case of certain hepatic intoxications [8, 18].

Our biochemical results are, in fact, in agreement with those of K e m m e l m e i e r and B r a c h t [14]. They found that, in the perfused liver, 0.1—0.5 mM mefenamic acid (another NSAID) determined an increase of glycogenolysis and glycolysis as well as an inhibition of glucose formation (although, starting from fructose, only). Combined with an increase of oxygen consumption (also observed), the results were explained in terms of the primary uncoupling action of mefenamic acid.

Also, B r a s s and G a r r i t y [5], working on isolated hepatocytes, find that certain NSAIDs (such as meclofenamate, ibuprofen and indomethacin) intensify glycolysis. I t i n o s e *et al.* [12] observe the stimulation of GGL/GL and the inhibition of GNG by the analgesic drug acetaminophen. However, in this last case, the respiration of isolated mitochondria was not stimulated but inhibited. Such an observation is not totally unexpected. Even in our case, at the highest concentrations tested (0.1 mM for diclofenac and 10 mM for aspirin) a reversal of the stimulating tendency of respiration was noted (see Fig. 3, for ex.). This is in line with our previous observations [23, 24] that unspecific uncouplers sti-

mulate the respiration at relatively low and moderate concentrations but inhibit it at higher concentrations.

According to Weissmann [29], NSAIDs would be expected to alter membrane processes that depend on the overall mobility of the membrane lipids. At the same time, it is known that oxidative phosphorylation is a membrane-related process whose efficiency is critically dependent of the relative impermeability of the inner mitochondrial membrane (see [16] as a primary source). However, oxidative phosphorylation is not the only process which depends on perfect integrity of membrane, although it may be the primary factor for many of them. Calcium entry into the mitochondria depends on the membrane potential generated by respiration, while calcium extrusion from cytosol on the ATP (also generated by respiration), which is needed for the work of Ca-ATPases involved in this process (see [16]). The increase of intracellular calcium and the activation of protein kinase C are necessary steps in signal transduction for the aggregation of the inflammation cells and the release of the inflammatory factors, while the capacity of the lipophilic NSAIDs to insert into lipid bilayer could result in the disruption of signal transduction through plasma membrane (see [2]). However, from what is known about the effects of the uncoupling agents on mitochondria (*i.e.*, energy dissipation and calcium release), the addition of NSAIDs would result in the increase of cytosolic calcium and (consequently) in the stimulation and not the reduction of the inflammation process. Also, the increase of cytosolic calcium could explain the stimulation of GGL/GL observed in our study. According to [11] and other literature data, glycogen phosphorylase (the key enzyme in glycogen breakdown) is stimulated by increased calcium concentration. Thus, the influence of the uncoupling phenomenon on glucose metabolism may not be so direct as described by us, but probably dependent (or, additionally dependent) of calcium.

Certainly, one can argue that NSAIDs also have a more specific effect on particular proteins (or, more generally, mechanisms) involved in signal transduction at the level of plasmalemma, a process which presumably occurs at lower concentrations than those needed for the uncoupling of mitochondria, which is a secondary (toxic) effect. Indeed, the concentrations of NSAIDs used in the present study (as well as in similar ones) are generally considered higher than the pharmacological doses. But, as already mentioned, and as we shall elaborate in our accompanying paper [26], the uncoupling effect begins at lower concentrations, *i.e.* in the range where the anti-inflammatory effect occurs. In addition, the fact that we do not find irreversible effects for a relatively long period of perfusion (30 min) suggests that such effects are probably not the consequence of a strong covalent binding. Therefore, in agreement with Itinose *et al.* [12], we consider that weak and reversible chemical interactions may be important for the mechanism of action of NSAIDs. What this mechanism is and how it can be reconciled with the uncoupling effect should be a matter of future concern.

The complexity of this problem is also illustrated by some recent observations regarding the involvement of certain prostaglandins in the regulation of carbohydrate metabolism. Thus, it has been shown that, in the perfused liver, prostaglandins are able to stimulate both glycogenolysis [7] and gluconeogenesis [10, 22]. However, we are not sure of the relevance of such observations for our own studies and it is certainly difficult to make a definite connection between the two sets of results without actually measuring prostaglandin concentrations in the perfused liver from our experiments.

Conclusions. It is clear, we hope, that our study was not specifically designed for testing any hypothesis regarding the anti-inflammatory mechanism of NSAIDs, but, in addition to the information concerning the effects of such two important drugs (diclofenac and aspirin) on glucose metabolism, our results have also raised uncomfortable questions regarding the compatibility of the anti-inflammatory action with the uncoupling effect of these drugs. If anything else, our observation that aspirin and diclofenac have a notably uncoupling effect, in the concentration range used in clinical treatments, may have an important toxicological relevance.

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UNELE ASPECTE HISTOCHIMICE ŞI BIOCHIMICE ALE ACŢUNII UNUI EXTRACT VEGETAL ÎN TOXICOZA CU TETRACLORURĂ DE CARBON

MIRCEA A. RUSU*, NICOLAE BUCUR* şi MIRCEA TĂMAŞ**

SUMMARY. — Some Histochemical and Biochemical Aspects of the Action of a Plant Extract in Toxicosis with Carbon Tetrachloride. Young, about 40-day-old rats were intoxicated with carbon tetrachloride (CCl_4) and treated with an alcoholic extract of *Calendula officinalis* for one week. Then, some of the animals were sacrificed and samples of liver and blood were taken. The rest of them did not received CCl_4 any more; they were given only *C. officinalis* extract for one week. Thereafter, these animals were also sacrificed and their liver and blood sampled. Histological (hematoxylin-eosine staining), histoenzymological (lactate dehydrogenase, succinate dehydrogenase, cytochrome oxidase, Mg-dependent adenosine triphosphatase), histochemical (Sudan black staining for lipids) and biochemical (seric glutamic-pyruvic and glutamic-oxaloacetic transaminases) analyses were carried out. The results have shown that CCl_4 intoxication manifested itself in severe liver injuries (increase in seric glutamic-pyruvic transaminase activity, necrosis, cellular balooning, decrease in histoenzimatic activity, etc.). The *C. officinalis* extract had some favourable effects, diminishing hepatocytolysis and liver injuries.

Toxicozele sunt frecvente în ultimele decenii ca urmare a apariţiei unor xenobiotice cu structuri noi (medicamente, pesticide, adjuvanţi alimentari etc.) utilizate neadecvat sau a utilizării intensive a unor xenobiotice mai vechi. Organismul întreg suferă de acest impact, dar organul cel mai sensibil este ficatul, deoarece aici se metabolizează majoritatea xenobioticelor. În consecinţă, cresc afecţiunile hepatice cauzate de unele substanţe, şi se amplifică studiile care abordează acest subiect, combinate cu obţinerea unor preparate naturale sau de sinteză care să controleze această situaţie.

În cadrul cercetărilor experimentale de toxicoză hepatică, o substanţă frecvent utilizată este tetraclorura de carbon (CCl_4) [1, 5, 8, 11]. Noi am utilizat în experimentul nostru CCl_4 pentru a realiza starea de toxicoză hepatică, din mai multe motive: este o substanţă toxică poluantă, fiind prezentă în mediul înconjurător al omului (industrie, agricultură, curăţătorii chimice etc.), are un tropism hepatic evident, produce efecte precise şi sigure, uşor reproductibile. Remediile utilizate în afecţiunile hepatice sunt de mai multe feluri, printre care şi produsele vegetale din categoria extractelor, inclusiv extractul de *Calendula officinalis* pe care ne propunem să-l testăm.

Reabilitatea fitoterapiei în contemporaneitate pune noi mijloace de tratament neconvenţionale la îndemâna terapeutului [3].

* *Institutul de Cercetări Biologice, 3400 Cluj-Napoca, România*
** *Universitatea de Medicină şi Farmacie, Facultatea de Farmacie, 3400 Cluj-Napoca, România*

Material și metode. Am utilizat în experiment șobolani tineri masculi din rasa Wistar alb, în greutate medie de 70±10 g, în vârstă de 40 zile. Animalele au fost crescute în condiții zooigienice corespunzătoare. Substanțele — CCl_4 și extractul de *Calendula* — au fost administrate prin gavaj, intragastric, animalele fiind „à jeun". Pentru obținerea extractului alcoolic s-a utilizat *Calendulae flos cum calicibus* provenit de la soiul „Gigant Pacific", cultivat la Universitatea de Științe Agricole Cluj-Napoca, în anul 1992. Extractul a fost analizat din punct de vedere fizico-chimic și s-a standardizat în conținut de derivați fenilpropanici (acid cafeic) — 150 mg/100 g și flavonoide — 120 mg/100 g.

Experimentul s-a desfășurat în două faze.

Faza I. S-a efectuat o intoxicare de 7 zile cu CCl_4 și un tratament concomitent cu extractul de *Calendula*. S-au format următoarele loturi: *lotul martor*, notat cu *M;* fiecare șobolan din acest lot a primit câte 0,4 ml/100 g greutate corporală ulei de floarea soarelui, zilnic, timp de 7 zile;

— *lotul intoxicat cu* CCl_4, notat cu *C;* fiecare șobolan din acest lot a primit câte 0,1 ml CCl_4 în 0,3 ml ulei de floarea soarelui/100 g greutate corporală, zilnic timp de 7 zile;

— *lotul intoxicat cu* CCl_4 *și tratat cu extract de Calendula*, notat cu *CE;* fiecare șobolan din acest lot a primit câte 0,1 ml CCl_4 în 0,3 ml ulei de floarea soarelui/100 g greutate corporală; după cca 30 minute s-a administrat câte 1,4 ml/100 g greutate corporală de extract de *Calendula*, zilnic, timp de 7 zile; a urmat prima sacrificare pentru recoltare de ficat și sânge.

Faza a II-a. S-a întrerupt intoxicarea cu CCl_4, administrându-se în continuare numai extract de *Calendula*, zilnic, timp de 7 zile în dozele de mai sus. A urmat a doua sacrificare pentru recoltare de ficat și sânge de la cele 3 loturi. Șobolanii au fost sacrificați prin secționare cervicală. Din sângele recoltat s-a determinat activitatea transaminazei glutamico-piruvice (GPT) și a transaminazei glutamico-oxalacetice (GOT) (metoda Reitman — Frankel). Din ficatul recoltat, unele fragmente au fost prelucrate pentru histologie, secțiunile fiind colorate cu hematoxilin-eozină (HE). Alte fragmente de ficat au fost congelate în azot lichid și apoi secționate la criotomul tip SLEE. Secțiunile au servit la determinarea activității următoarelor enzime [9]: lactatdehidrogenaza (LDH) succinatdehidrogenaza (SDH), citocromoxidaza (CyOx), adenozintrifosfataza Mg-dependentă (ATP-aza). Tot pe secțiunile obținute la criotom s-au evidențiat lipidele totale colorate cu Sudan negru [9].

Rezultate și discuții. *Faza I.* Șobolanii au fost sacrificați după 7 zile de intoxicație cu CCl_4 și tratament cu extract de *Calendula*. Supraviețuirea nu a fost afectată la loturile experimentale. În schimb, se modifică aspectul macroscopic al ficatului în ceea ce privește culoarea (este mai deschisă) și suprafața ficatului care este în general brobonată la lotul *C* și în mai mică măsură la lotul *CE*.

— *Indici biochimici.* În ser nivelul activității GPT crește cu valori foarte mari la lotul *C* (476%), în timp ce la lotul *CE* crește cu 323%.

Nivelul activității GOT crește cu 56% la lotul *C* și cu 43% la lotul *CE* (Tabel 1).

— *Indici histologici, histoenzimologici și histochimici. Coloratia cu hematoxilin-eozină.* La lotul *M* se observă imaginea histologică normală a ficatului de șobolan. La cei din lotul *C* sunt remarcate leziuni majore care se caracterizează prin necroze centrolobulare, numeroase celule balonizate (suferind de degenerare balonizantă) izolate sau care confluează disecând unele zone din parenchimul hepatic, imagini frecvente de distrofie clară și granulară, precum și numeroase lipide în zonele centrolobulare, care dovedesc existența steatozei hepatice. La șobolanii din lotul *CE* se observă aceeași categorie de modificări, dar mai reduse. Ac-

Tabel 1

Faza I. Activitatea transaminazelor serice (GPT și GOT)
(Valorile sunt exprimate în μg acid piruvic/ml ser)

	GPT			GOT		
	M	C	CE	M	C	CE
X	75,3	434,2	319	294	459	423
n	7	8	8	7	8	8
ES±	5,0	44,3	31,5	7,7	26,5	12,2
D%	100	576	423	100	156	143,9
t	—	8,0	7,6	—	6,1	8,9
p	—	<0,001	<0,001	—	<0,001	<0,001

tivitatea LDH, SDH, CyOx, ATP-azei este mai redusă la lotul *C* și în măsură mai mică este afectată la lotul *CE*. Diminuarea activității enzimatice este rezultatul scăderii intensității reacțiilor enzimatice și al distrugerii unor zone de parenchim hepatic mai mult sau mai puțin extinse în special ca un efect al necrobiozelor și al degenerării balonizante.

Coloratia cu Sudan negru pentru lipide. Evidențiază existența unei steatoze mai accentuate la lotul *C*.

Faza a II-a. Șobolanii au fost sacrificați după 7 zile de la prima sacrificare. Supraviețuirea lor nu a fost afectată. Se mai remarcă la nivel macroscopic, la lotul *C*, o ușoară brobonare a suprafeței ficatului, culoarea acestuia este cvasinormală.

— *Indici biochimici.* În ser nivelul activității GPT rămâne mai crescut cu 44% la lotul *C* și cu 31% la lotul *CE* în comparație cu lotul *M*. Activitatea GOT nu se modifică semnificativ (Tabel 2).

— *Indici histologici, histoenzimologici și histochimici. Coloratia cu hematoxilin-eozină.* La lotul *C* structura histologică a ficatului este mult ameliorată în comparație cu faza precedentă. Astfel se remarcă

Tabel 2

Faza a II-a. Activitatea transaminazelor serice (GPT și GOT)
(Valorile sunt exprimate în μg acid piruvic/ml ser)

	GPT			GOT		
	M	C	CE	M	C	CE
X	99,4	143,2	131,2	282	283	278
n	7	8	8	7	8	8
ES±	6,2	6,1	8,6	12,7	15	14,1
D%	100	144	132	100	100	98,6
t	—	5	3,02	—	0,2	0,2
p	—	<0,001	<0,01	—	NS	NS

reducerea numărului de celule necrotice, precum și atenuarea marcată a fenomenului de balonizare celulară — zonele cu aceste leziuni fiind mai reduse ca extindere. Au mai rămas prezente fenomene distrofice (clară și granulară). La lotul *CE* modificările sunt mai diminuate în comparație cu lotul *C*. Activitatea enzimelor studiate rămâne la lotul *C* sub nivelul lotului martor. La lotul *CE* activitatea enzimatică este apropiată de lotul *M*. Coloratia Sudan negru pentru lipide nu mai evidențiază steatoză hepatică.

Din prezentarea rezultatelor se observă modificări lezionale severe, consecutiv intoxicației cu CCl_4 de la nivelul microscopic până la nivel citologic și enzimatic. Cele mai grave leziuni apar în faza I experimentală. Remarcăm îndeosebi creșterea foarte puternică a transaminazelor serice, în special GPT, ceea ce confirmă rezultatele din literatură [1, 11]. GPT este de altfel o enzimă caracteristică ficatului [4]. De asemenea, tabloul citologic normal al ficatului este modificat, remarcându-se îndeosebi celule necrobiotice și cele suferind de degenerare balonizantă. Activitatea enzimelor studiate este mai redusă în comparație cu lotul martor. Lotul *CE* prezintă acelasi tip de leziuni, dar mai reduse ca și la lotul *C*. Amintim că nivelul GTP este cu 32% mai mic decât la lotul *C*. În faza a II-a în condițiile administrării numai a extractului de *Calendula officinalis* la amândouă loturile experimentale, tabloul lezional se reduce semnificativ ca o consecință a unui intens și complex proces de refacere [2]. Acest proces de refacere la toate nivelurile analizate macroscopic, citologic, enzimatic (de exemplu, nivelul GPT scade da cca. 10 ori în comparație cu faza I) este mai intens la lotul *CE*. Mecanismul de acțiune al CCl_4 este cunoscut în bună măsură. Citocromul P450 2El este responsabil de metabolizarea CCl_4 la radicalul liber — CCl_3 [6, 7, 10, 12] care produce peroxidarea lipidelor și lezarea membranelor intra- și extracelulare, influențând negativ inclusiv hemeostazia enzimatică. Mogoș și Sitcai [8] consideră intoxicația cu CCl_4 ca un model util pentru hepatita toxică asemănătoare cu hepatita umană.

În ceea ce privește acțiunea extractului de *Calendula* sunt puține date referitoare la acțiunea hepatotropă a acestei plante. Cercetările noastre indică o certă acțiune de protecție în cazul intoxicației cu CCl_4. Rezultatele cele mai bune se referă la o diminuare a hepatocitolizei și o atenuare a leziunilor histologice. În faza a II-a, extractul vegetal facilitează refacerea. Conținutul în derivați fenilpropanici (de tipul acidului cafeic), precum și în flavonoide poate fi cauza efectului protector. Acești compuși pot să acționeze împotriva radicalilor liberi rezultați din bioactivarea CCl_4, facilitând astfel rolul extractului hidroalcoolic de *Caendula officinalis*.

Concluzii. Administrarea extractului hidroalcoolic de *Calendula officinalis* produce la nivelul ficatului de șobolani intoxicați cu CCl_4 unele efecte favorabile caracterizate în special prin reducerea hepatocitolizei și a leziunilor hepatice.

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EVOLUTION OF THE ENZYMATIC POTENTIAL IN LEAD AND ZINC MINE SPOILS SUBMITTED TO BIOLOGICAL RECULTIVATION

DANIELA PAŞCA*, ŞTEFAN KISS, MIHAIL DRAGAN-BULARDA**, RADU CRIŞAN* and VASILE MUNTEAN***

SUMMARY. — Seven enzymatic and nonenzymatic catalytic activities were determined in the 0-10-cm layer of 14 small and two large recultivation plots installed in 1987 and 1988, respectively on lead and zinc mine spoils in Rodna (Bistriţa-Năsăud district, Romania). The plots as well as the untreated control places in the vicinity of plots and a native soil at the foot of the spoil dump were enzymologically analysed 19 times during the 1987 (1988) — 1994 period. The analytical data served for calculation of the annual enzymatic indicator considered as an index of the biological quality of spoils (and soils) and, implicitly, as an index of the recultivation efficiency. These long-term experiments confirmed the findings made after 1—2 years of recultivation: the most important measure for creation of an enzymatic potential in spoils is their covering with soil. The best results were registered in a large (50-m^2) recultivation plot which had been covered with a 10-cm soil layer, fertilised with NPK and inoculated with a small amount of spontaneously revegetated 15-year-old spoils contining seeds of plants as well as microorganisms adapted to the toxic environment of lead and zinc mine spoils.

Enzymological evaluation of the measures applied for biological recultivation of lead and zinc mine spoils in Rodna (Bistriţa-Năsăud district, Romania) was carried out since 1987 and 1988, respectively.

On June 24, 1987, 14 small (7-m^2) recultivation plots were installed on the 2-year-old terrace VIII (plots 1—6), on the 7-year-old terrace V (plots 7—10) and on the 10-year-old terrace III (plots 11—14) of the spoil dump. On July 5, 1988, two large (50-m^2) recultivation plots were installed on the 5-year-old terrace VI (plots I and II). The treatments applied to plots are briefly specified in Table 1. Untreated places in the vicinity of plots served as controls. A native, soddy soil at the foot of the spoil dump also served for comparison. Detailed description of the recultivation experiments is given in [4—6].

In the June 1987 — October 1989 period, the 0-10-cm layer of plots was analysed enzymologically. Based on the results obtained, a complex treatment (covering with soil, fertilization with NPK and sowing of a grass-legume mixture) was recommended for rapid recultivation of raw and young spoils [4, 5]. Efficiency of this complex treatment was confirmed by enzymological analyses of spoils sampled from the 0-10-, 10-20- and 20-30-cm layers of plots [6].

* *Biological Research Institute, 3400 Cluj, Romania*
** *Babeş-Bolyai University, Department of Plant Biology, 3400 Cluj, Romania*

Table 1

Treatments applied to recultivation plots

Date of the installation of plots	Terrace no. and age	Aspect*	Plot no.	Treatment**
June 24, 1987	VIII (2 years)	SW	1	S + FYM + NPK + GL
			2	FYM + NPK + GL
			3	NPK
			C 1–3	No (control)
		SE	4	S + FYM + NPK + GL
			5	FYM + NPK + GL
			6	NPK
			C 4–6	No (control)
	V (7 years)	SW	7	NPK + GL
			8	NPK
			C 7,8	No (control)
		SE	9	NPK + GL
			10	NPK
			C 9,10	No (control)
	III (10 years)	SW	11	NPK + GL
			12	NPK
			C 11,12	No (control)
		SE	13	NPK + GL
			14	NPK
			C 13,14	No (control)
July 5, 1988	VI (5 years)	SW	I	S + NPK + GL
			C I	No (control)
		SE	II	S + NPK + Spoil-15
			C II	No (control)

* *SW* — South-western. *SE* — South-eastern.
** *S* — Covering with soil. *FYM* — Farmyard manuring. *NPK* — Fertilisation with NPK. *GL* — Sowing of grass-legume mixture. *Spoil-15* — Inoculation of spontaneously revegetated, 15-year-old spoils.

The present paper deals with the evolution of the enzymatic potential in the 0-10-cm layer of spoil plots during the 1987 (1988) — 1994 period.

Materials and methods. For enzymological analyses, spoil samples were taken from the plots and control places 19 times, at the following dates: June 24 and September 7, 1987; July 5 and October 10, 1988; July 5 and October 18, 1989; April 20, June 28 and October 12, 1990; March 25 and August 2, 1991; May 25 and August 28, 1992; May 6, July 19 and September 22, 1993; and May 11, August 1 and October 12, 1994. In 1989 and in the next years the native soil was also sampled.

In all samples, 7 enzymatic and nonenzymatic catalytic activities, namely invertase activity in nonbuffered and buffered (3 M acetate buffer; pH 5.6) re-

action mixtures [3], phosphatase activity [7], catalase and nonezymatic H_2O_2-splitting activities [2], and actual and potential dehydrogenase activities [1] were analysed. The analytical data served for calculation of the enzymatic indicator considered as an index of the biological quality for spoils (and soils) and, implicitly, as an index of the recultivation efficiency [5]. The experimental plots, control places and the native soil were characterized by annual enzymatic indicators.

Results. The results obtained are presented in Figs. 1—4.

Fig. 1, comprising the enzymatic indicators of the small plots installed on terrace VIII, shows that the enzymatic potential of the com-

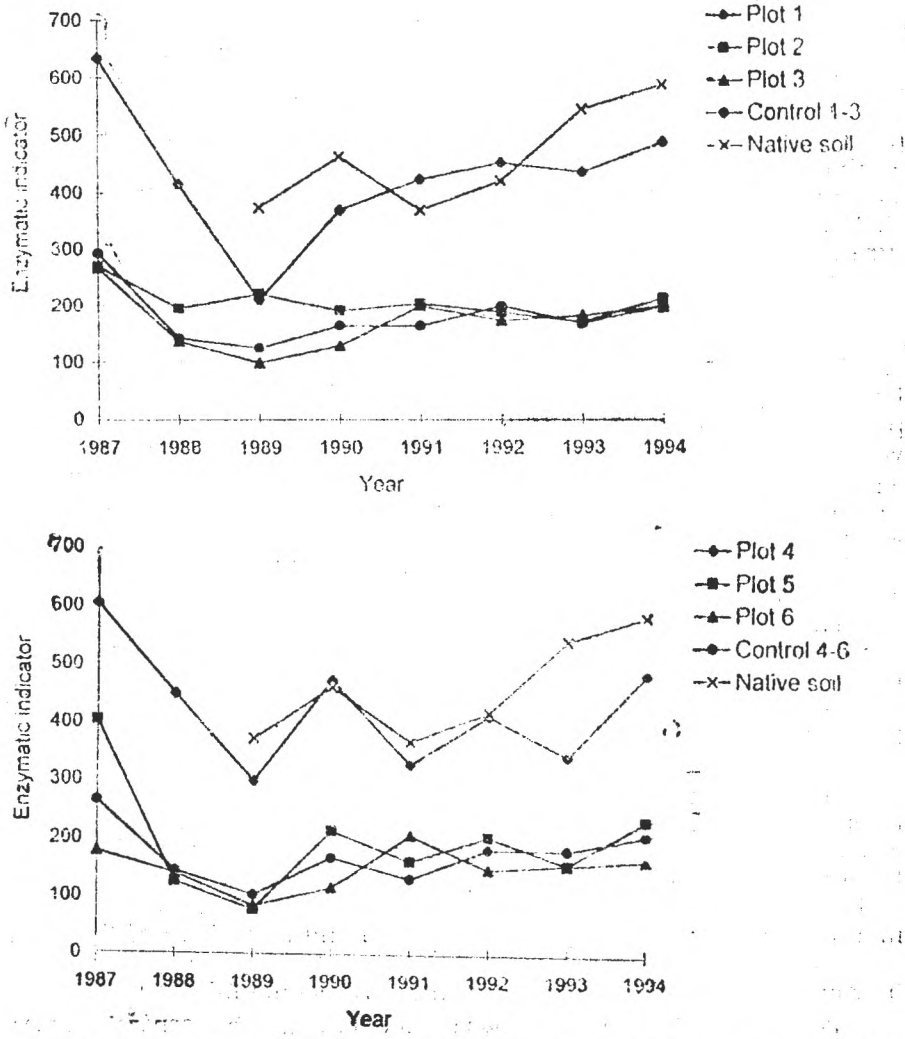

Fig. 1. *Evolution of the enzymatic potential in spoil plots installed on terrace VIII.*

plexly treated plots 1 and 4 is much higher than that of the plots not covered with soil (plots 2, 3 and 5, 6) and of the control places (C 1-3 and C 4-6), and is even close to that of the native soil.

Amplitude of the annual variation of enzymatic potential is larger in the complexly treated plots and native soil than in the other plots and control places. In the 1987—1989 period, the enzymatic potential manifested a general trend to decrease, which was followed, in the 1990—1994 period, by an increasing trend in the complexly treated plots and native soil and by levelling off in the other plots and control places.

Comparison of plots 1-3 (south-western aspect) with plots 4-6 (south-eastern aspect) reveals no evident aspect-dependent variation in enzymatic potential, excepting a single case: in the 1987—1989 period, decrease of enzymatic potential was more pronunced in plot 1 than in plot 4.

One can see from Figs. 2 and 3 that the enzymatic potential in plots 7-10 and 11-14 on terraces V and III, respectively is much lower than that of the native soil. As these plots were not covered with soil, the high enzymatic potential registered in plots 1 and 4 on terrace VIII indicates again that covering with soil in the most important measure for creation of an enzymatic potential in spoils.

Figs. 2 and 3 also show that in the 1987—1989 period a decrease occured in the enzymatic potential of plots 7-14, too. Later, the enzymatic potential slightly increased or levelled off. Plots 11—14 installed on the 10-year-old terrace III exhibited a little higher enzymatic potential than plots 7-10 installed on the 7-year-old terrace V. As the plots on both terraces received the same treatment (NPK fertilization with or without sowing of grass-legume mixture), one can state that the age of spoils positively influenced the formation of their enzymatic potential. The untreated, control places were always the least enzyme-active.

The aspect-dependent variation of the enzymatic potential on terraces V and III is negligibly low.

The results obtained in the two large plots I and II installed on the 5-year-old terrace VI are illustrated in Fig. 4. It is evident from this figure that the enzymatic potential of these plots always exceeded that of the native soil. Decrease in the enzymatic potential of these plots in 1989 was followed by a marked increasing trend in the 1990—1994 period. These findings, like those registred in plots 1 and 4 prove the role played by covering with soil in the creation of enzymatic potential in spoils. Plot II, covered with soil, NPK-fertilized and inoculated with a small amount of spontaneously revegetated, 15-year-old spoils was a little more enzyme-active than plot I, covered with soil, NPK-fertilized and sown with grass-legume mixture. This means that the plants and microorganism from the spontaneous flora and microflora of the old spoils contributed more efficiently to the formation of enzymatic potential in younger spoils than did the sown grass-legume mixture.

The enzymatic potential was very low in the control places and showed little variation in the 1990—1994 period.

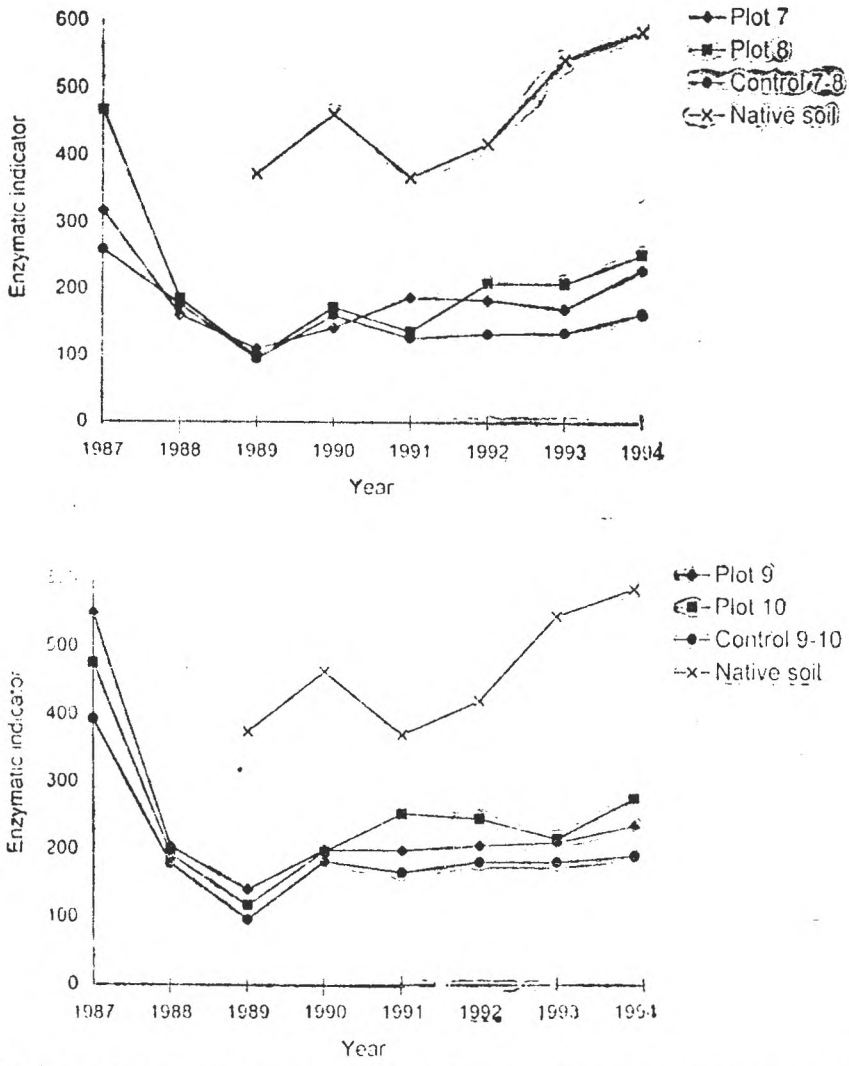

Fig. 2. *Evolution of the enzymatic potential in spoil plots installed on terrace V.*

An aspect-dependent variation in the enzymatic potential can not be considered because the two large plots received different treatments.

The treatments applied to the small and large plots in the 1987—1989 period as described in [4-6] was not followed by any treatments in the next years, *i.e.* the plots received no fertilizers and they were not sown and moistened artificially. Nevertheless, the spoils retained an enzymatic potential which was highest in the soil-covered plots.

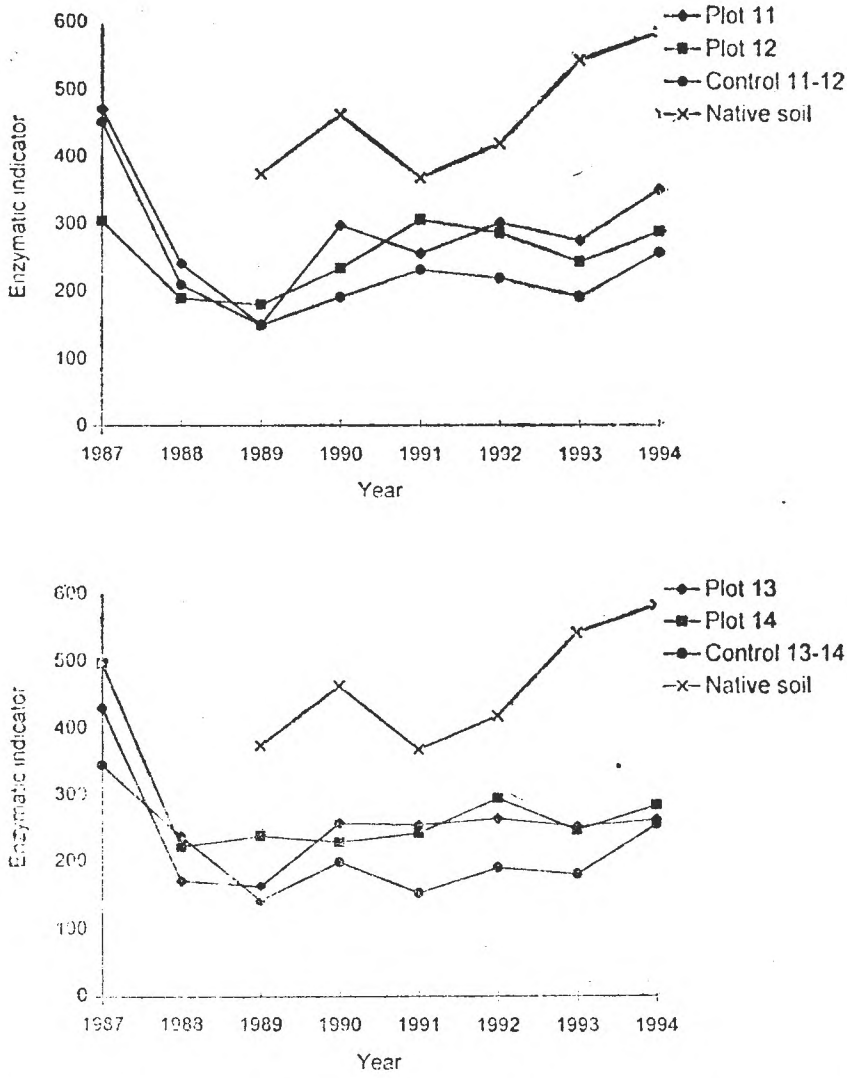

Fig. 3. *Evolution of the enzymatic potential in spoil plots installed on terrace III.*

Conclusions. 1. Long-term recultivation experiments on lead and zinc mine spoils confirmed the findings made in short-term experiments, namely that the most important measure for creation of an enzymatic potential in spoils is their covering with soil.

2. The best results were registered in a large (50-m^2) recultivation plot which had been covered with a 10-cm soil layer, fertilized with

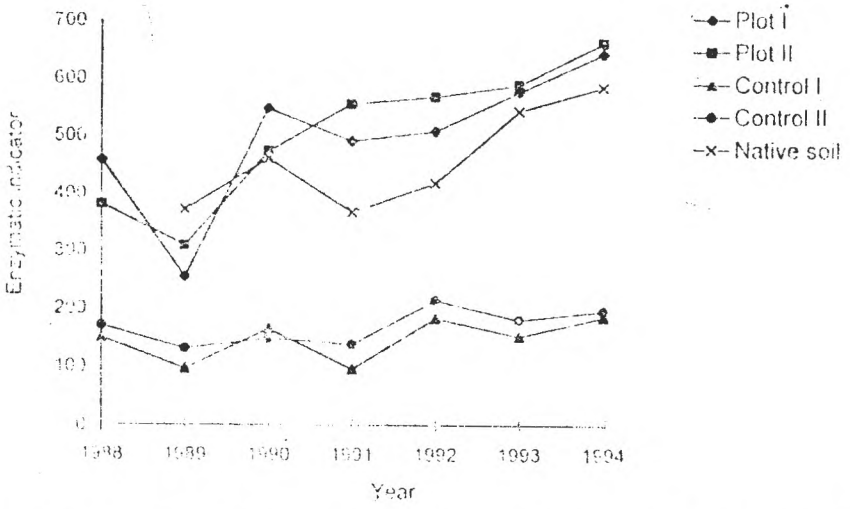

Fig. 4. *Evolution of the enzymatic potential in spoil plots installed on terrace VI.*

NPK and inoculated with a small amount of spontaneously revegetated, 15-year-old spoils containing seeds of plants as well as microorganisms adapted to the toxic environment of lead and zinc mine spoils.

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ENZYMATIC SYNTHESIS OF β-ALKYL-D-FRUCTOFURANOSIDES IN SOIL

ANA CHISTOL* and ŞTEFAN KISS*

SUMMARY. — Reaction mixtures were prepared from air-dried samples of a leached chernozem, an aqueous sucrose solution and 35% (v/v) *n*-propanol or equimolecular quantities of other alcohols (methanol, ethanol, isopropanol and glycerol). Following incubation at 37°C, the liquid phase of the reaction mixtures was analysed by means of circular paper chromatography. The spots on chromatograms were revealed with reagents of reducing sugars or of free and combined ketoses. The results proved that both hydrolytic and synthetic reactions catalysed by soil sucrase took place in aqueous-organic solvent systems. Both water and methanol, ethanol and *n*-propanol served as acceptors of the fructosyl residue transferred from sucrose by soil sucrase, leading to the formation of free fructose (and glucose) and of β-alkyl-D-fructofuranosides, respectively. Amounts of the β-alkyl-D-fructofuranosides formed showed the order: β-methyl-D-fructofuranoside > β-ethyl-D-fructofuranoside > β-*n*-propyl-D-fructofuranoside, and each increased with prolongation of the incubation time. Isopropanol and glycerol were not found to be acceptors of the fructosyl residue transferable from sucrose by soil sucrase.

Soil oligases are able to catalyse not only hydrolytic reactions, but synthetic ones, too. Thus, the reaction mixtures prepared from soil, an aqueous solution of enzyme substrate and toluene (antiseptic), incubated for different periods of time, then analysed by means of paper chromatography, showed the presence of hydrolytic product(s) and also of compounds having smaller R_F values and higher molecular weights than the substrate. These synthetic reactions resulted from transferring of a part of the substrate moieties not on water molecules, but on intact, unsplit substrate molecules or on newly synthesised molecules.

Both hydrolytic and synthetic activities were described with the following soil oligases: maltase (substrate: maltose) [1-4, 8-10]; sucrase (substrate: sucrose) [2, 3, 6-8]; cellobiase (substrate: cellobiose or arbutin) [2-4]; and lactase (substrate: lactose) [2, 3, 10]. The synthetic products of the reactions catalysed by soil oligases are always oligosaccharides.

Occurrence in soil of a polysaccharide (levan)-synthesising enzyme (levan sucrase) was also proved [5, 7]: the reaction mixtures (soil + toluene + aqueous sucrose solution), following their incubation, contained a nonmigrating compoud (R_F =O) of high molecular weight, consisting of fructose units and identified as levan.

Synthetic reactions of soil oligases in aqueous-organic solvent systems were studied only in the case of sucrase. During incubation of

* *Babeş-Bolyai University, Department of Plant Biology, 3400 Cluj, Romania*

reaction mixtures prepared from 2 g of air-dried soil plus 0.5 ml of toluene plus 10 ml of a 10% (w/v) sucrose solution in 15% (v/v) aqueous methanol, a fast-migrating (great R_F). fructose-containing nonreducing compoud appeared, besides the hydrolytic products (fructose and glucose). The new compound was identified as β-methyl-D-fructofuranoside. It was lacking in reaction mixtures to which no methanol was added [8]. Under similar conditions, β-ethyl-D-fructofuranoside was produced in reaction mixtures consisting of 3 g of air-dried soil, 2 ml of toluene and a 10% (w/v) sucrose solution in 50 or 60% aqueous ethanol [6, 7].

Performing synthetic enzymatic reactions in organic solvents or in mixed aqueous-organic solvent mixtures is, nowadays, gaining more and more importance especially for the pharmaceutical and food industries. Reactions in organic solvents are also carried out for enzymatic conversion of some organic pollutants (phenols and amines, including the carcinogenic amines), present in industrial wastewaters, into easily removable compounds (see for example the review by [11]).

The progress in investigations on enzymatic reactions in organic solvents reactualises the problem of soil enzymatic activities in aqueous-organic solvent systems. For our present study, the starting point was a finding by Ryu *et al.* [12], according to which a commercial horseradish peroxidase preparation retained its oxidative and polymerising activity on the phenolic compound tested (bisphenol A) even in reaction mixtures containing *n*-propanol in a high proportion (35% v/v).

Materials and methods. A leached chernozem was studied. The soil samples were taken from the 5-15-cm depth, then allowed to dry on air. The air-dried soil was ground in a mortar and sieved to pass a 2-mm screen. Two experiments were carried out.

In the first experiment, transformation of sucrose in soil in the presence of 35% (v/v) *n*-propanol was studied; *n*-propanol was tested as fructosyl residue acceptor. Reaction mixtures without *n*-propanol or sucrose or soil served as controls. Composition of the reaction mixtures is presented in Table 1.

In the second experiment, *n*-propanol as fructosyl residue acceptor was compared with other alcohols used in equimolecular quantities. Table 2 specifies the alcohols studied and the composition of reaction mixtures.

Table 1

Composition of the reaction mixtures in the first experiment

Reaction mixture no.	Soil (g)	Toluene (ml)	10% (w/v) aqueous sucrose solution (ml)	*n*-Propanol (ml)	Distilled water (ml)
1	5	—	6.5	3.5	—
2	5	2	6.5	—	3.5
3	5	—	—	3.5	6.5
4	5	2	—	—	10
5	—	—	6.5	3.5	—
6	—	2	6.5	—	3.5
7	—	—	—	3.5	6.5

Table 2

Composition of the reaction mixtures in the second experiment

Reaction mixture no.	Soil (g)	Toluene (ml)	10% (w/v) aqueous sucrose solution (ml)	Methanol (ml)	Ethanol (ml)	*n*-Propanol (ml)	Isopropanol (ml)	Glycerol (ml)	Distilled water (ml)
1	5	—	6.5	1.8	—	—	—	—	1.7
2	5	—	6.5	—	2.6	—	—	—	0.9
3	5	—	6.5	—	—	3.4	—	—	0.1
4	5	—	6.5	—	—	—	3.5	—	—
5	5	—	6.5	—	—	—	—	3.3	0.2
6	5	2	6.5	—	—	—	—	—	3.5
7	—	—	6.5	1.8	—	—	—	—	1.7
8	—	—	6.5	—	2.6	—	—	—	0.9
9	—	—	6.5	—	—	3.4	—	—	0.1
10	—	—	6.5	—	—	—	3.5	—	—
11	—	—	6.5	—	—	—	—	3.3	0.2
12	—	2	6.5	—	—	—	—	—	3.5

In both experiments, the reaction mixtures were incubated at 37°C. After 5—21 days of incubation, the liquid phase of reaction mixtures was analysed by means of circular paper chromatography. Paper used: Whatman I. Volume of the liquid analysed: 5 μl. After drying, the chromatograms were developed in a solvent mixture consisting of *n*-propanol, ethyl acetate and water (6:1:3 v/v/v); the development took place at room temperature and lasted 2 hours. For revelation of the spots of reducing sugars, *i.e.* fructose and glucose, resulted from the sucrase-catalysed hydrolysis of sucrose, a reagent containing $AgNO_3$ [14] was used. Spots of the free and combined ketoses (fructose, sucrose, β-alkyl-D-fructofuranosides, oligofructosides, levan) were revealed with a reagent containing urea and H_3PO_4 [13].

Results. In both experiments, the chromatograms revealed with the $AgNO_3$-containing reagent showed the presence of reducing sugars in the reaction mixtures prepared from soil and sucrose without or with alcohol addition. This means that the soil sucrase manifested its hydrolytic activity on sucrose in both absence and presence of alcohols.

The chromatograms in Fig. 1 were obtained in the first experiment. They were revealed with the urea-H_3PO_4 reagent detecting the spots of ketoses. One can see from this figure that in the reaction mixture 1 (1') (soil + sucrose + *n*-propanol) a fast-migrating, fructose-containing compound (*a*), β-*n*-propyl-D-fructofuranoside, has formed. In other words, soil sucrase could use *n*-propanol as an acceptor of the fructosyl residue of sucrose. In the reaction mixture 2 (2'), products of reactions catalysed by two soil enzymes were present: fructose (*b*) and oligofructosides (*d*) formed due to the soil sucrase and levan (*e*) produced from sucrose by levan sucrase. The control reaction mixtures prepared without sucrose (3, 3', 4, 4') were free of ketoses, whereas the other controls (sucrose + *n*-propanol and sucrose + toluene) (5, 5', 6, 6') contained only sucrose. Thus, chemical (nonenzymatic) formation of β-*n*-propyl-D-fructofurano-

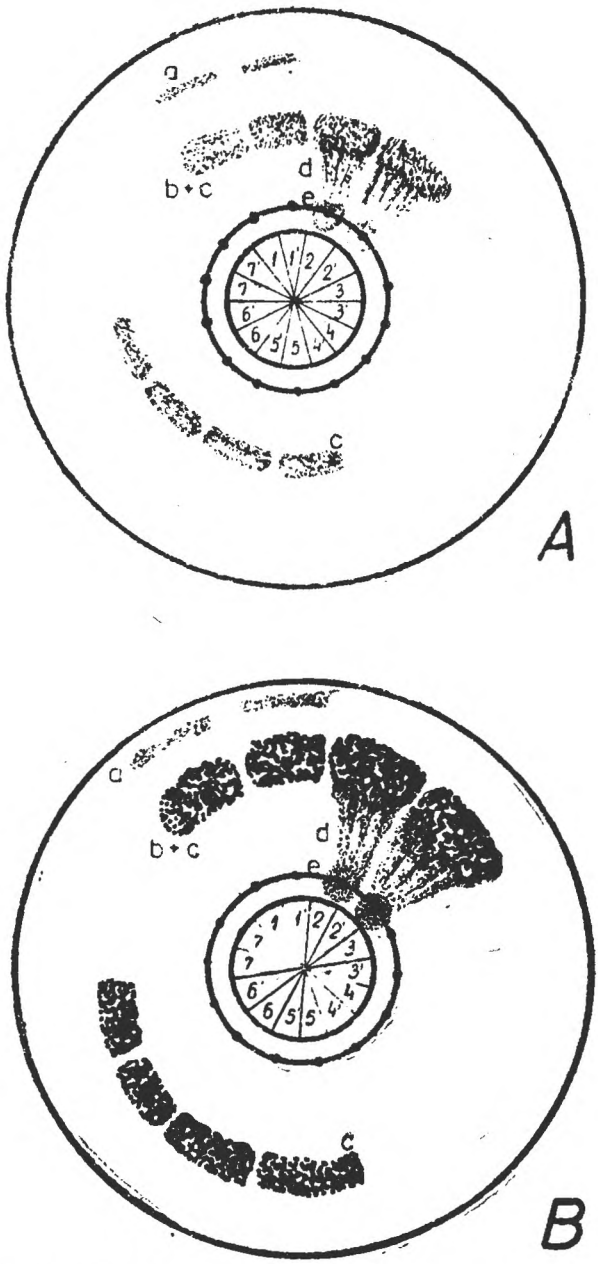

Fig. 1. *Enzymatic synthesis of β-n-propyl-D-fructofuranoside in soil. A* — 5-day incubation *B* — 21-day incubation. 1—7 — Reaction mixtures 1—7: see Table 1. 1′—7′ — Repeated reaction mixtures 1—7. *a* — β-*n*-Propyl-D-fructofuranoside. *b* — Fructose. *c* — Sucrose. *d* — Oligofructosides. *e* — Levan.

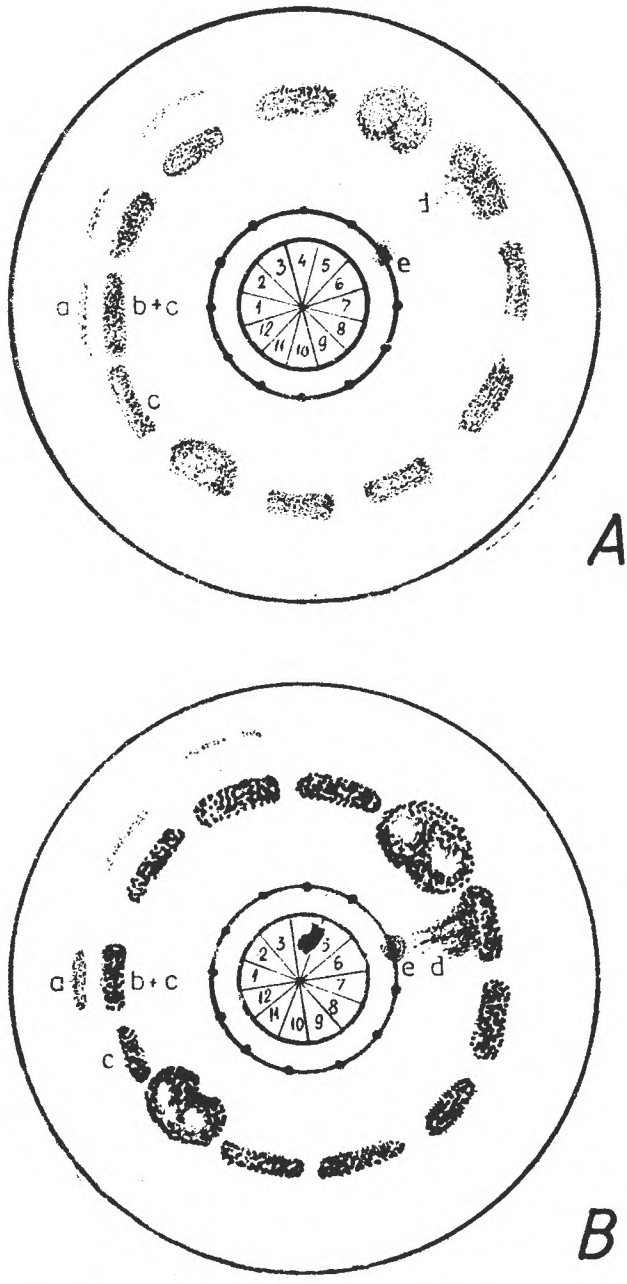

Fig. 2. *Enzymatic synthesis of β-alkyl-D-fructofuranosides in soil. A* — 7-day incubation. *B* — 14-day incubation. 1—12 — Reaction mixtures 1—12: see Table 2. *a* — β-Alkyl-D-**-fructofuranosides** (β-methyl-, β-ethyl- and β-*n*-propyl-D-fructofuranosides). *b—e* — See Fig. 1.

side should be excluded. The control reaction mixture 7 (7') (no soil, no sucrose) was, of course, free of ketoses.

From comparison of chromatograms A and B one can deduce that prolongation of the incubation time from 5 to 21 days led to an evident increase in the amount of each synthetic product.

Two of the chromatograms obtained in the second experiment and revealed with the urea-H_3PO_4 reagent (Fig. 2) show that of the 5 alcohols studied (methanol, ethanol, *n*-propanol, isopropanol and glycerol) only the first three served as acceptors of the fructosyl residue of sucrose (reaction mixtures 1—3), determining the formation of β-methyl, β-ethyl- and β-*n*-propyl-D-fructofuranoside, respectively. Their amounts decreased in this order. This finding means that the transfer activity of soil sucrase decreased with increasing length of the carbon chain in the fructosyl acceptor alcohols, because the alcohols were used in equimolecular quantities. It should be mentioned again that the branched alcohol, isopropanol and the trivalent alcohol, glycerol in the reaction mixtures 4 and 5, respectively, did not give rise to any sucrase-synthesised products. As expected ,the hydrolytic and synthetic products in reaction mixture 6 (soil + toluene + sucrose) were fructose (and glucose), oligofructosides and levan. The control reaction mixtures 7-12 behaved like reaction mixtures 5 and 6 in the first experiment.

It results from the comparison of chromatograms A and B that increasing the incubation time from 7 to 14 days brought about the formation of synthetic products in higher amounts.

Conclusions. 1. Both hydrolytic and synthetic reactions catalysed by soil sucrase took place in aqueous-organic solvent systems.

2. In reaction mixtures prepared from air-dried soil, aqueous sucrose solution and 35% (v/v) *n*-propanol, acceptors of the fructosyl residue transferred from sucrose by soil sucrase were water and *n*-propanol, leading to the formation of free fructose (and glucose) and of β-*n*-propyl-D-fructofuranoside.

3. In the reaction mixtures, to which methanol, ethanol and *n*-propanol were added in equimolecular quantities, amounts of the β-alkyl-D-fructofuranosides formed showed the order: β-methyl-D-fructofuranoside > β-ethyl-D-fructofuranoside > β-*n*-propyl-D-fructofuranoside, and each increased with prolongation of the incubation time.

4. Isopropanol and glycerol did not serve as acceptors of the fructosyl residue transferable from sucrose by soil sucrase.

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RECENZII

G. Z a r n e a, **Tratat de microbiologie generală. V. Bazele teoretice ale ecologiei microorganismelor. Microorganismele şi mediile lor naturale** *(Treatise of General Microbiology. V. Theoretical Bases of Microbial Ecology. The Microorganisms and Their Natural Environments)*, Editura Academiei Române, Bucureşti, 1994, 1078 pages (including 387 figures and 76 tables) and 36 plates with electron micrographs and photographs enclosed.

Volumes I, II, III and IV of the *Tratat de microbiologie generală* appeared in 1983, 1984, 1986 and 1990, respectively and were reviewed in this journal (Stud. Univ. Babeş-Bolyai, Biol., 1985, 30, 75—76; 1986, 31 (1), 67—70; 1987, 32 (2), 92—94 and 1990, 35 (2), 103—107, respectively).

Volume V is the first monographic work on microbial ecology published in our country and one of the 4—5 similar works appeared in the universal literature.

Volume V consists of two parts, each comprising many chapters, subchapters and sections.

The chapter titles within the two parts of the work are specified below.

Part 1. Theoretical Bases of Microbial Ecology: Microbial ecosystems. Role of microorganisms in the ecosystem (pp. 21—38); Adaptation of microorganisms to the ecosystem (pp. 39—42); The concepts of habitat and niche (pp. 43—53); Populations and communities of microorganisms (pp. 54—58); Ecological succession (pp. 59—71); Diversity of the communities of microorganisms (pp. 72—85); Nutrition and growth of microorganisms in natural environments (pp. 86—105); Ecological energetics (pp. 106—129); Dispersion of microorganisms in nature (pp. 130—153); Influence of interfaces on the microorganisms. Microbial biofilms (pp. 154—166); Influence of environmental conditions. Extremophilic microorganisms (pp. 167—201); Viability, survival and longevity of microorganisms (pp. 202—217); Quantification of the occurrence and activity of microorganisms in nature (pp. 219—250); Interactions among the populations of microorganisms (pp. 251—284); Symbiosis (pp. 285—366); Mycorrhizae (pp.367—391); The symbiosis cyanobacteria-fungi or algae-fungi. Lichens (pp. 392—408); Parasitism (pp. 409—428); Predation (pp. 429—448); Pathogenic microorganisms (pp. 449—489); Bacterial toxins (pp. 490—550).

Part 2. The Microorganisms and Their Natural Environments: Ecosphere (pp. 553—557); The atmo-ecosphere as a habitat and major medium for dispersion of microorganisms in nature (pp. 558— 574); Microbiology of cosmic space (pp. 575—579); The soil as a natural environment for microorganisms. Structure and dynamics of the populations of soil microorganisms (pp. 581—661); Hydroecosphere. Structure and dynamics of the populations of aquatic microorganisms (pp. 663—749); Normal microbiota of the animal organisms (pp.751—792); Role of microorganisms in the global cycle of matter in nature (pp. 793—834); Biological degradation of the plant constituents (pp. 835—899); Geological activity of microorganisms (pp 901—940); Microbial biodegradation and biodeterioration (pp. 941—1006); Microbiology of wastewaters (pp. 1007—1050).

There are 780 books, monographs, review and original articles listed in the Selected bibliography (pp. 1051—1078).

The defining characteristics of Volume V are the same as those of Volumes I—IV, namely up-to-date and comprehensive scientific content, richness in very representative tables, figures, electron micrographs and photographs, clarity of the descriptions, attractive style, creation of the adequate Romanian terminology for the terms appeared in the universal microbiological literature in the last decades.

One can deduce even from the chapter titles that Volume V of the *Tratat de microbiologie generală* is a basic source of information for students and experts in many fundamental and applied sciences, *e.g.* soil, aquatic, industrial, medical and veterinary microbiology, ecology, biochemistry, plant and animal physiology, phytopathology, botany, zoology,

hydrology, geology, medicine, agriculture environmental engineering.

My suggestion already advanced for Volumes I—IV to be translated at least into English is also valid for Volume V. I am convinced that the English translation of Professor G. Zarnea's *Tratat de microbiologie generală*, completed with subject and author indices, will be a best-seller all over the world, due to the high qualities of this work.

ŞTEFAN KISS

Metody pochvennoi mikrobiologii i biokhimii *(Methods of Soil Microbiology and Biochemistry)*, Pod red. (Edited by) Professor D. G. Zvyagintsev, Izdatel'stvo Moskovskogo Universiteta, Moscow, 1991, 304 pages with 64 figures and 14 tables in the text.

The first edition of this work (224 pages) was published in 1980 (see our review in Stud. Univ. Babeş-Bolyai, Biol., 1982, 27 (1), 78—79). For the present, second edition, the work was revised and enlarged. New methods developed and older methods improved during the 1980—1990 period are also included into the second edition. At the same time, methods that lost their importance were omitted. The first edition, elaborated by 4 investigators from the Soil Biology Department of the Moscow State University, comprised 5 chapters, whereas the second edition, to which 17 investigators contributed from the same Department, consists of 7 chapters. A key for identification of the most characteristic soil bacteria at genus level was also added, in form of an appendix, to the second edition.

The book contains the detailed description of a great number of methods used in the complex research of natural and artificial ecosystems (biogeocoenoses), of the effects of anthropic interventions (fertilization, liming, pesticide application, irrigation, drainage, pollution, etc.) on the soil life and fertility. Methods for isolation and study of different groups of soil microorganisms are also described in detail. Some of the methods are original, being worked out by the authors of the book (methods for: studying soil microorganisms by means of fluorescent microscopy; microbiological analysis of the rhizoplane; determination of N_2 fixation under field conditions; analysis of nucleic acids, ATP, muramic acid in soil, etc.).

As mentioned above, the second edition of the book consists of 7 chapters.

Chapter 1 deals with sampling of soils for microbiological analyses.

Chapter 2, entitled „Mehods for observation and enumeration of soil microorganisms and determination of their biomass", is divided into three subchapters: Direct microscopic methods; Biochemical methods for determination of soil microbial biomass; Methods for studying, directly in the soil, the microbial associations and the interrelationships among different groups of organisms.

Within Chapter 3, „Isolation, enumeration and identification of soil microorganisms", the following topics are dealt with: Preparation of laboratory vessels and materials for microbiological inoculation; Methods for isolation and enumeration of bacteria in soil; Methods for isolation of micromycetes from the rhizosphere and rhizoplane; Analysis of the physiological functions of bacteria; Methods for isolation and identification of soil bacteria; Methods for isolation and cultivation of anaerobic bacteria; Isolation and enumeration of soil micromycetes; Methods for identification of micromycetes; Methods for estimating the number and biomass of yeasts in soils; Taxonomic treatment of the collection of soil yeast isolates.

Chapter 4 is devoted to „Methods for studying dynamics of microbial populations in soil" and comprises three subchapters: Immune fluorescent microscopy; Variants resistant to antibiotics; Method of membrane chambers.

Chapter 5 describes „Methods for studying microbial successions in soil (structure of the complex of soil microorganisms)". The subchapters are entitled „Method for determination of the structure of the complex of soil fungi and actinomycetes according to their radial growth rate" and „Determination of the structure of the complexes of soil micromycetes".

Chapter 6, „Methods for determination of the biological activity of soils", includes: Methods for studying nitrogen fixation in soil; Methods for studying denitrification in soil; Determination of the nitrifying activity of soil; Method of the initiated microbial community (in starch-amended and incubated soil samp-

les, the species composition and structure of the amylolytic microbial community are determined); Method for determining microbial toxicosis of soil; Method of cellophane membranes; Enzyme activity of soil; Field methods for determination of the CO_2 gas exchange in the „soil-plant-atmosphere" system; Determination of the nitrogen fixation activity under field conditions; Determination of the denitrification activity under field conditions; Applicational methods.

In Chater 7, „Determination of physiologically active microbial substances in soil", methods are described for determination of amino acids, nucleic acids (DNA and RNA), ATP and muramic acid.

The bibliographical list comprises 69 titles, of which 42 are in Russian and 27 in English.

The book can be characterized by a series of qualities: comprehensive and up-to-date information, logical grouping of the methods, clarity of the descriptions, attractive style.

The book is addressed not only to students and specialists in soil microbiology and biochemistry as it is a valuable methodological book also for students and specialists in other environmental sciences. For students and specialists not speaking Russian, I warmly recommend translation of the book at least into English.

ŞTEFAN KISS

Bodenbiologie in Österreich *(Soil Biology in Austria)*, Eigentümer, Herausgeber und Verlager (Owner, Editor and Publisher): Österreichische Bodenkundliche Gesellschaft (Austrian Soil Science Society), Wien, 1994, 442 pages with 153 figures, 52 tables and 4 maps in the text.

The book comprises the Proceedings of the Conference of the Austrian Society of Soil Biology, held in Linz on 4th and 5th of November 1993. It was published as issue No. 48—49/1994 of the Mitteilungen der Österreichischen Bodenkundlichen Gesellschaft. It includes 20 oral communications, 23 poster presentations and the list of the 107 participants.

The first 4 communications are reviews of the development of soil microbiology and soil zoology in Austria (F. Schinner and F. Schaller, respectively) and in Germany (J. C. G. Ottow and W. Dunger, respectively). The other communications deal with original investigations. We specify their titles and authors: Microbial, nonenzymatic phosphorus mobilisation from inorganic phosphates (P. Illmer); Effects of fertilisation on the soil mesofauna of different forest stands in Austria (H. Kopeszki); Seasonal variation of microbiological activities in a black soil with different tillage (K. Böhm, E. Kandeler, W. E. H. Blum); Field studies on the bioindicator potential of soil protozoa: experiences from Austria (E. Aescht); Soil zoological investigations in agricultural landscapes of Austria (Burgenland, Upper Austria) (E. Meyer); Monitoring of ectomycorrhizae (R. Pöder, B. Pernfuß); Enzyme activity determinations within the framework of the Austrian Forest Soil Monitoring Programme — arylsulphatase (B. Philipp, F. Mutsch, E. Kandeler, R. Maier); Soil Monitoring Programme in Upper Austria — substrate-induced respiration, N-mineralisation, phosphatase (R. Öhlinger); Forest melioration in the Calcareous Alps; use of intact soil cores for estimation of the leaching of nitrate and ammonium (H. Insam); Biological soil purification (E. Bauer, C. Pennerstorfer, E. Kandeler, R. Braun); Influence of easily mobilisable heavy metals on the activity of soil microorganisms (E. Lummerstorfer, E. Kandeler, O. Horak); Availability of trace metals for soil animals (earthworms) on the example of an industrial site (M. Palzenberger, H. Pohla); Field ecological study of ciliates *(Protozoa)* in heavy metal-polluted soils (A. Berthold); Interactions between soil mesofauna and microflora in field mesocosms. I. Technique and experimental design (C. Kampichler, A. Bruckner, R. Bauer, E. Kandeler), II. Recolonisation of defaunated mesocosms by oribatids, collembolans and enchytraeids (A. Bruckner, R. Bauer, C. Kampichler, E. Kandeler), III. Biomass and nutrient metabolism of soil microorganisms (E. Kandeler, B. Winter, C. Kampichler, A. Bruckner, R. Bauer).

The poster presentations, like the oral communications, cover a wide range of topics: Microbiological activity in soil aggregates of different stability (S. Aichinger, E. Kandeler); Soil biological activities in mixed vegetable cultures (G. Bachmann, M. Müllebner); Influence of nitrogen input from the atmosphere on the microbiological processes in forest

soils (G. Bauernfeind, F. Schinner); Effects of organic fertilisers in combination with magnesite on soil microbiological parameters in a spruce stand in Upper Austria (M. Berreck, K. Haselwandter); The *Diplura* of Vienna (E. Christian); Biochemical analyses of an ammonium nitrate-fertilised soil in pot experiment (G. Gemeinhardt); Influence of magnesite, organic and mineral fertilisers on the soil enzymatic activities in a spruce forest (B. Girschick, S. Zechmeister-Boltenstern); Research of mycorrhizae in Austrian forests (F. Göbl); Nitrous oxide emission through denitrification in an acid forest ecosystem (M. Henrich, K. Haselwandter); A microcosm experiment on effects of forest fertilisation on nitrogen leaching and soil microbial properties (H. Insam, A. Palojärvi); Investigations for analysis of an epigeic collembolan coenosis (C. Kampichler); Roughness of the surface of soil pores and its influence on the available habitat space for microarthropods (C. Kampichler, M. Hauser); Collembolans as active bioindicators of soil contaminations (H. Kopeszki); Applicability of rock meals for improvement of nutrient availability in different forest soils (long-term investigations) (R. Kuhnert-Finkernagel, W. v. Mersi, F. Schinner); On the soil fauna in forests of Voralberg (Austria) — stand and effects of rock meal applications (E. Meyer, K. H. Steinberger); Microbial activities and biomass along an altitudinal gradient in the Northern Calcareous Alps (A. Rangger H. Insam, K. Haselwandter); Different nitrogen dynamics in arable and grassland soils (J. Reschenhofer, W. Strobl, R. Öhlinger); Soil type, farming system and soil biological parameters: Ursprung-Elixhausen (G. Smejkal); Soil microbiological studies in long-term experimental plots in the Czech Republic (J. Stana, T. Sevcik, S. Maly); Bacterial potassium mobilisation from illitic clay minerals (W. v. Mersi, F. Schinner); Fungal potassium mobilisation from illitic clay minerals (W. v. Mersi, F. Schinner); Investigation of soil microbiological parameters during the conversion from conventional to biological farming system at Lobau (I. Wieshofer); Soil biological activity measurements in a contaminated beech stand in the Vienna Forest (R. Zehner, A. Mentler, M. Pfeffer, W. E. H. Blum).

It is evident from the book that the soil biological investigations in Austria have reached a high level; they bring valuable contributions to the study of most major fundamental and applied aspects of soil biology. Foundation of the Austrian Society for Soil Biology in 1991 and publication of the second edition of the „Bodenbiologische Arbeitsmethoden" by F. Schinner, R. Öhlinger, E. Kandeler and R. Margesin in 1993 (see our review on this methodological book in Studia Univ. Babeş-Bolyai, Biologia, 1993, *38* (1—2), 135—136) also reflect the development of soil biology in Austria.

ŞTEFAN KISS

Restoring Farmland after Coal. The Bryngwyn Project *(Restaurarea terenului agricol după [exploatare de] cărbune. Proiectul Bryngwyn),* Edited by (Sub red.) John Scullion, British Coal Opencast şi The University of Wales, Institute of Biological Sciences, Aberystwyth, 1994, VI+104 pagini cu 42 tabele şi 31 grafice în text şi 16 fotografii color în anexă.

Proiectul a fost elaborat în 1997 când s-a înfiinţat o gospodărie agricolă din 94 hectare de teren pe care s-a încheiat exploatarea la zi a cărbunelui şi din 30 hectare de teren învecinat care nu a fost afectat de exploatarea minieră. Gospodăria a cuprins şi farmul Bryngwyn. În 1984, suprafaţa gospodăriei a crescut cu 21 hectare de teren folosit mai înainte pentru exploatare de cărbune. Gospodăria este situată în partea sudică a Ţării Galilor. Înaintea extracţiei cărbunelui, solul de suprafaţă, cel din straturile subiacente şi materialele de descopertare au fost îndepărtate şi depozitate în grămezi separate, iar după încheierea extracţiei cărbunelui, ele au fost reintroduse în ordine inversă. Atât solul cât şi materialele de descopertare se caracterizează printr-o textură fină, din care cauză ele uşor devin compacte.

În carte sunt descrise experimentele de recultivare de lungă durată (17 ani) efectuate la Bryngwyn, mai ales în condiţii de câmp şi, în măsură mai mică, în casă de vegetaţie. S-au aplicat metode de recultivare cu plante agricole, respectiv cu plante forestiere. S-au efectuat studii pedologice, agrochimice, botanice, zoologice, statistice şi economice.

Rezultatele înregistrate şi concluziile

trase cu aplicabilitate directă pentru gospodăria de la Bryngwyn, dar considerăm că unele din concluzii au valabilitate generală. Citäm mai jos câteva din aceste concluzii.

Depozitarea în grămadă a solului de suprafaţă duce la înrăutăţirea proprietăţilor lui structurale şi biologice. Astfel, râmele, care joacă un joc important în structurarea solului, sunt eliminate din interiorul grămezii din cauza anoxiei. Dacă depozitarea solului de suprafaţă în grămadă nu poate fi evitată, atunci, pentru a fi folosit în recultivare, solul trebuie îndepărtat în mai multe etape, dar întotdeauna numai din stratul superior al grămezii, lăsând apoi partea superioară a grămezii rămase să se recolonizeze cu râmele din zonele învecinate.

Drenarea efectivă a terenului supus recultivării este esenţială.

Elementul nutritiv principal, a cărui carenţă limitează creşterea plantelor pe terenul supus recultivării, este azotul.

Orice întreprindere agricolă, situată pe terenuri recultivate după exploatări miniere, este viabilă, din punct de vedere economic, dacă ea cuprinde şi terenuri neafectate de exploatările miniere, în proporţie de cel puţin 20% faţă de suprafaţa terenurilor recultivate.

Cartea editată de Dr. J. Scullion confirmă că evoluţia solurilor de mină spre soluri fertile este un proces foarte lent, din care cauză urmărirea acestui proces necesită cercetări de lungă durată.

ŞTEFAN KISS

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