

alanine and serine per  $10^8$  cells  $\text{h}^{-1}$ , as well as 4–6  $\mu\text{moles}$  arginine. Doubling arginine in the medium *Hexamita* led to a reduction in glycogen storage, glucose, alanine and serine catabolism. No regulatory mechanism was evident from available information.

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*Giardia intestinalis* Needs Glucose to Complete Its Life Cycle. J.G. ZALITIS and D.S. CROSS, School of Biochemistry and Molecular General, University of New South Wales, Sydney, Australia.

Previous studies have shown that *Giardia* trophozoites and cysts contained glycogen equivalent to glucose of about 4 and 7  $\mu\text{moles}$  per  $10^8$  cells, respectively. We have studied the origin and utilisation of cyst glycogen. The yield, glycogen content and viability of the cysts were dependent on glucose in the encystation medium. The optimal glucose concentration was 15 mM or higher. Encystation of trophozoites in 1 mM glucose resulted in a 80% decrease in cyst numbers, with low glycogen content and abwater at 37°C. In normal cysts, incubated at 25 or 37°C, glycogen decreased exponentially,  $t_{1/2}$  being 36 and 20 hours, respectively. Cyst numbers remained constant till the glycogen had been depleted. Normal cysts started to swell or rupture in water after about 100 hours at 37°C. By adding  $^{14}\text{C}$  glucose at 2-hour time intervals to encysting trophozoites, maximum labeling of cyst glycogen occurred between 0–4 hours, decreasing to negligible after 12 hours. From these results, it can be inferred that cyst glycogen was formed from glucose in encysting trophozoites. *Giardia* trophozoites grew well in low glucose medium, as judged by cell number increase and  $^3\text{H}$  thymidine incorporation. During growth in low glucose medium, trophozoite glycogen content progressively decreased to about 10% of normal in 48 hours, when medium glucose was undetectable. Prolonged culture in low-glucose medium had no effect on trophozoites other than to decrease the rate of cell division or encystation. Normal cysts obtained by exposing encysting trophozoites to  $^{14}\text{C}$  glucose had 90% of the label in glycogen. Incubation of cysts at 37°C resulted in a decrease in  $^{14}\text{C}$  glycogen with a qualitative increase in  $^{14}\text{CO}_2$  and acetate. Cysts appear to metabolise glycogen via glycolysis to maintain their cell integrity.

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The *Cryptosporidium parvum* Lactate Dehydrogenase Gene. G. ZHU\*, M.J. MARCHEWKA and J.S. KEITHLY, Wadsworth Center, New York State Department of Health, Albany NY.

Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) are two enzymes present in archaeobacteria, eubacteria and eukaryotes. In eukaryotic cells, LDH is localized in cytosol, while MDH isoforms can co-exist in the cytosol and organelles, including mitochondria, chloroplasts and peroxisomes. The LDH/MDH protein sequences are highly conserved and phylogenetically related. Both LDH and MDH are 2-ketoacid: NAD(P) oxidoreductases, sharing similar tertiary structures. Despite all of these structural and functional similarities, most LDH/MDH enzymes are highly specific to their substrates, i.e., either lactate or malate, but not both. The substrate specificity is correlated with the presence of a single amino acid in the catalytic site: all MDH possess a positively charged arginine, whereas all LDH contain an uncharged residue, e.g., glutamine at the same position. Both LDH and MDH enzymatic activities have been reported for *Cryptosporidium parvum*. Prior to this

study, molecular characterization of these enzymes was lacking. Here we present both molecular and phylogenetic analyses of a *C. parvum* LDH homologue (CpLDH1). The CpLDH1 gene encodes 330 amino acids, which are highly conserved with other LDH/MDH, including those from its apicomplexan relatives, *Plasmodium falciparum* and *Toxoplasma gondii*. The key residue at the active site of CpLDH1 is a non-polar glycine, rather than a charged arginine, strongly suggesting that lactate is the preferred substrate for this enzyme. RT-PCR indicates the expression of CpLDH1 occurs both in sporozoites and intracellular parasites. Interestingly, phylogenetic analyses suggest that all apicomplexan LDH, including CpLDH1, are a sister group to both eubacterial and archaeobacterial MDH. This (apicomplexan LDH and bacterial MDH) clade is then a sister group to other eukaryotic and prokaryotic LDH. The significance of this mixed lineage for apicomplexan LDH is not fully understood, but warrants further investigation.

## Czech Section Society of Protozoologists 30th Annual Meeting April 25–28, 2000 (Abstracts 45–53)

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Immune Response of the Host to Infection with the Microsporidian, *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen, 1923. P. BRAUNFUCHSOVA, J. SALAT, Institute of Parasitology, Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic.

In the present study, the immune response of immunocompetent BALB/c mice and severe combined immunodeficient (SCID) mice to intraperitoneal infection with the microsporidian, *Encephalitozoon cuniculi* was analyzed. The production of three cytokines, interferon gamma (IFN-gama), interleukin 10 (IL-10) and interleukin 12 (IL-12), was measured. High levels of IFN-gama were detected in ex-vivo cultures of peritoneal exudate cells (PEC) of BALB/c mice, a lower, but earlier IFN-gama response was observed in PEC from SCID mice. The early IL-10 response was detected in ex-vivo cultures of splenocytes from BALB/c but not from SCID mice, explaining a delay in the IFN-gama response in BALB/c mice. The higher amount of spores in peritoneal macrophages was observed during the infection of SCID mice that develop a lethal infection. The populations of natural killer (NK) cells of both mice strains were activated by the infection. Duration and rate of NK activity differed between these strains. Production of IFN-gama correlated with NK activity. Production of specific antibodies was demonstrated from the 9th day after infection. Cytotoxic T-lymphocytes (CTLs) were generated in vitro by incubation of immune splenocytes with irradiated *E. cuniculi* spores. These CTLs were able to kill infected syngeneic macrophages. The results of this study support the theory of the essential role of IFN-gama and specific CTL activity for host protection.

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Phylogenetic Position of Some Trichomonad Species Isolated in Vitro from Different Animals. I. CEPICKA, V. HAMPL, J. KULDA and J. FLEGR, Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic.

Many trichomonad species described so far from different animals have been inadequately described and their taxonomic position is doubtful. In a project aimed at taxonomic revision

and phylogenetic relationships of some parabasalid groups were examined over 200 specimens from 90 species of mammals, birds, reptiles, amphibians and snails and obtained 60 isolates of trichomonads from faeces, rectum, caecum and oral cavity of 40 host species. Trichomonads belonged into 7 genera: *Monocercomonas* (2 species), *Hypotrichomonas* (1 sp.), *Trichomitus* (3 sp.), *Tritrichomonas* (2 sp.), *Trichomonas* (2 sp.), *Pentatrichomonas* (1 sp.) and *Tetratrichomonas* (more than 10 species). The gene for 5.8S rRNA with the flanking areas ITS1 and ITS2 of 10 strains of *Tetratrichomonas* from different species of Bovidae, Suidae and Tayassuidae, one strain from an African elephant and two strains of *Trichomitus* from different lizards was amplified, cloned, sequenced and analysed using Maximum parsimony and Neighbor-joining tree constructing methods. Tetratrichomonads from ruminants and from African elephant created one clade, but differed morphologically from *T. buttreyi* (the only well described *Tetratrichomonas* inhabiting ruminants and pigs). Similarities within this clade varied from 67.6% to 86.6%. As similarities between four distinct species of the genus *Trichomonas* varied between 86% and 89%, these tetratrichomonads probably belong to several separate species. *Tetratrichomonas gallinarum* formed the sister branch of this clade. The *Tetratrichomonas* strain from *Chelydra serpentina* which was morphologically similar to *T. brumpti* from land turtles formed another clade with *T. limacis* and *T. prowazeki*.

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Relationships of Flagellates of the Genus *Monocercomonas* from Different Host Species. V. HAMPL, I. CEPICKA, J. TACHEZY and J. FLEGR, Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic.

*Monocercomonas grassii* is widely distributed genus of gut parasites from vertebrates and insects. Most species were described from reptiles, but their taxonomic validity as well as the phylogenetic position of this genus in the evolution of the order Trichomonadida remains unclear. We studied the relationship of one isolate of *Monocercomonas ruminantium* from cow and 5 isolates of *Monocercomonas* sp. from reptiles (*Varanus exanthematicus* (VAR-1), *Tropidophis melanurus* (R183), *Natrix sipedon* (ATCC 502210), *Python regius* (PYR 1-1), *Eumeces* sp. (EUMM)). The sequences of 5.8S rRNA with the flanking areas ITS1 and ITS2 (5.8S rRNA) of all strains were determined. On the basis of these sequences the phylogenetic tree was constructed by the Neighbor-joining (NJ) and Maximum parsimony (MP) method. In both trees the strains from reptiles formed one branch with the bootstrap value of 100. The relatedness of EUMM, PYR 1-1, VAR 1 was very high (99.4% identity). On the other hand, the sequences of strains ATCC and R183 showed considerable differences from the previous strains (97.2% and 93% identity, respectively) as well as from each other (92.4% identity) indicating that both may represent different species. The isolate of *M. ruminantium* was clearly separated from the reptilian clade (85% identity). This isolate formed a monophyletic group with reptilian monocercomonads in the tree constructed by MP, but not in the NJ tree. In accordance with ultrastructural data as well as with the phylogenetic tree based on the 16S rRNA both branches of monocercomonads (reptilian clade and *M. ruminantium*) formed sister taxons to the genus *Tritrichomonas*.

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Increased Prevalence of Latent Toxoplasmosis in Victims of Traffic Accidents in Prague. J. HAVLICEK\*, P. KODYM \*\*, M. MALY \*\*\*

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*Toxoplasma gondii*, coccidian parasite whose frequency in man can reach 80% in some countries is known to influence the behaviour of its host. This is usually considered to be an evolutionary adaptation of the parasite aimed to increase the probability of its transmission from an intermediate host (any endothermic vertebrate) to the definitive host (cat) by predation. In human the latent toxoplasmosis results into the changes in personality profile as well as into the increase of reaction times measured in simple reaction time test. In modern society such behavioural changes can increase the probability that *Toxoplasma*-infected man fall victims of an traffic accident. In this study we compared the prevalence of latent toxoplasmosis in 103 victims of traffic accidents (car drivers and pedestrians hit by a car) with that obtained in two epidemiological surveys among inhabitants of the same area. We excluded from the analysis the persons who: did not actively participate in the accident, drank the alcohol or were not residents of Prague. Our results of age-stratified study suggest that the subjects with latent toxoplasmosis have 3.1 times higher probability of the accident than the uninfected controls (C.I.95 = 1.92-5.01; Mantel-Haenszel Chi-square = 25.219; p = 0.000). In the youngest age category (15-29 years) the relative risk of an accident for *Toxoplasma*-infected subjects was 4.35 (C.I.95 = 2.14-8.81). Because of its high prevalence in many developed countries, the latent toxoplasmosis might be in fact a very serious public health problem.

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Effect of Nisin Against the Cultures of Some Rumen Ciliates. S. KISIDAYOVA, A. LAUKOVA, and P. SIROKA, Academy of Sciences, Institute of Animal Physiology, Kosice, Slovakia.

The growth of the rumen ciliate *Entodinium caudatum* was not significantly affected by the nisin concentration of 0.01-0.4 mg/ml during short-term (5 day) treatment in vitro. Long-term (30 days) treatment decreased the growth of *Epidinium ecaudatum* f. *caudatum* et *ecaudatum* at the nisin dose of 0.1 mg/ml. However, the growth of *Entodinium caudatum* was not significantly influenced by the long-term (30 days) treatment at the nisin dose of 0.1 mg/ml. Nisin partly substituted gluten in the cultures as a source of amino acids. Inhibition of Gram-positive facultative anaerobic bacterial population of the cultures (lactobacili, enterococci, staphylococci, and amyolytic streptococci) was detected. The production of the total volatile fatty acids was significantly increased during long-term treatment of both cultures. The proportion of propionate was significantly increased (from 13 mol% to 30 mol%) on the account of acetate (from 73 mol% to 61 mol%).

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Opportunistic Properties of *Brachiola algerae* (syn. *Nosema algerae*) (Microspora, Protista) Demonstrated in the SCID Mice. B. KOUDELA\*,\*\* and J. VAVRA\*\*,\* \*Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno; \*\*Institute of Parasitology, Academy of Sciences of the Czech Republic, 370 05 Ceske Budejovice; \*\*\*De-

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The human isolate of *Brachiola algerae* (Visvesvara et al., J. Euk. Microbiol. 46, 10S, 1999) was inoculated intraperitoneally, intramuscularly and subcutaneously into Severe Combined Immunodeficient Mice. Spores were also applied to the eye surface and were fed to the mice host. No signs of disease were noted up to 60 days p.i., when the animals were autopsied and their organs examined by histology, using staining methods and Calcofluor M2R spore labeling. It was found that the microsporidium developed in the liver and peritoneal macrophages of the immunodeficient mouse host, but only after the ocular administration of spores. The infection of liver was severe and was manifested as hepatosplenomegaly and multifocal miliary necroses and granulomas containing parasites. No microsporidia were found in any other tissues. The identity of the parasite was confirmed by TEM which revealed characteristic tubulovesicular "secretory materials" on the plasma membrane of all developmental stages of *B. algerae* except sporoblasts and spores. Our observation is the first one demonstrating the opportunistic capability of *Brachiola algerae* to grow in mammalian viscera, far from the site of spore application. Eye can evidently serve as portal of entry for microsporidia into a mammalian organism. It is hypothesized that the physico-chemical milieu of the conjunctiva and cornea helped to adapt the originally "poikilothermic microsporidian" to the conditions within the homoiothermic organism.

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Trichomonads in Oral Cavity and Respiratory Tract of Humans: a New Species of Pathogenic *Tetratrichomonas*? K. KUTISOVA, J. KULDA and J. TACHEZY, Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic.

*Trichomonas tenax* regarded as a harmless commensal of human oral cavity. Although cases of pulmonary trichomoniasis have been described in patients with lung abscess, bronchiectasis or cancer disease, pathogenicity of *T. tenax* remains controversial. In addition, occasional infections of the respiratory tract of infants by genitourinary parasite *Trichomonas vaginalis* have been reported. The aim of present study was to distinguish *T. tenax* strains isolated from human oral cavity and bronchi as well as from other trichomonad species by two methods of DNA analysis: [1] PCR method based on random amplified polymorphic DNA (RAPD), and [2] nucleotide sequencing of ITS1–5.8rRNA–ITS2 region. Axenic strains of trichomonads isolated from oral cavity (5 strains) and from bronchi (5 strains) was a kind gift of Prof. J. Teras, Tallin, Estonia. The RAPD method using 9 random primers (UBC, University of British Columbia, Vancouver, Canada) provided complex patterns of amplified DNA fragments which divided Estonian strains into two distinct groups: "bronchial group" which consists of 5 bronchial and 2 oral strains, and "oral group" which was formed by remaining 3 oral strains. Both "bronchial" and "oral group" were distinct from *T. vaginalis*, *Pentatrichomonas hominis* and *Tritrichomonas foetus*. Comparison of nucleotide sequences encoding ITS1–5.8rRNA–ITS2 region (approximately 370 bp) showed consistent differences between strains of "bronchial" and "oral" groups. Three nucleotide substitutions and two deletions were observed in ITS1, two in 5.8rRNA and two in ITS2 region. These results together with RAPD analysis suggest that the "oral" and the "bronchial" groups represent different trichomonad populations or subspecies. Surprisingly, comparison of the sequences between Estonian strains and *T.*

*tenax* Hs-4:NIH (ATTC 30207) showed only 66 % similarity. Further analysis including sequences of *T. vaginalis*, *Trichomonas gallinae*, *Tetratrichomonas gallinarum*, *P. hominis*, *T. foetus* and *Tritrichomonas mobilensis* showed, that the Estonian strains are closely related to *T. gallinarum*. The sequence similarity between Estonian strains and *T. gallinarum* was 99 % and 95 % for bronchial and oral strains, respectively, while comparison with other species showed >70 % similarity. Morphological studies by means of light microscopy (protargol staining), and by electron microscopy confirmed that all Estonian strains belong to the genus *Tetratrichomonas*: the organisms possessed 4 anterior flagella, the recurrent flagellum extending as a free posterior flagellum behind the undulating membrane, and discoid parabasal apparatus. Our results suggest that the Estonian strains represent a new species of the genus *Tetratrichomonas* which can inhabit oral cavity and bronchi of humans. Future work will be focused to confirm presence of tetratrichomonads in cases of human respiratory tract infections.

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Heterogeneity of alpha- and beta-tubulin in microtubular systems of *Giardia intestinalis*. P. TESAROVA and E. NOHYNKOVA, Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, Studnickova 7, 128 00 Prague 2, Czech Republic.

It is well known that *Giardia intestinalis*, a bi-nucleated flagellate, possesses several cytoskeletal systems based on microtubules. Of these, flagellar axonemes and basal bodies represent permanent structures persisting during whole cell cycle, while occurrence of median body, adhesive disc and mitotic spindle is limited to certain phases of the cycle. We used a set of 15 monoclonal antibodies specific to different N- and C-terminal domains of alpha- and beta-tubulin, tyrosinated, acetylated and glutamylated alpha-tubulin, phosphoserine and phosphotyrosine to investigate occurrence and distribution of tubulin isoforms and posttranslational modifications in permanent and cell-cycle dependent microtubular structures. Two-dimensional gel analysis revealed at least ten isoforms of alpha- and three isoforms of beta-tubulin. Immunoblotting showed that all of alpha-tubulin isoforms were posttranslationally modified with different pattern of tyrosination, acetylation, glutamylation and phosphorylation, and indicated phosphorylation of dominant beta-tubulin isoform. Immunofluorescence staining showed different distribution of posttranslational modifications within *Giardia* cytoskeleton. Immunogold labeling revealed that tyrosinated tubulin was present on surface of outer doublets of axonemal microtubules, whereas no label was detected in central pair microtubules. In addition, flagella were decorated with antibodies recognizing the very C-terminal end of both, alpha- and beta-tubulin, which, in contrast, did not label cell-cycle dependent structures, namely mitotic spindle, median body and adhesive disk. In these, N-terminal ends of alpha- and beta-tubulin subunits were detected with domain-specific antibodies. Moreover, acetylation was the only posttranslational modification found in their microtubules. Our results indicate that microtubular systems of *Giardia* contain a significant amount of tubulin isoforms differing in posttranslational modifications as well as in subcellular localization which could reflect their specific, yet unknown functions.

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Species Composition and Vectors of Avian Trypanosomes—Molecular Evidence. J. VOTYPKA\*, \*\*, M. SVOBODOVA\*\*,



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About 100 species of avian trypanosomes (Kinetoplastida) have been named so far, however, most of them are considered to be synonyms. It remains unclear if each trypanosome species parasitizes a single host species or if it occurs in a wide spectrum of hosts. Mosquitoes, blackflies, hippoboscids and biting midges were suggested as vectors of avian trypanosomes. We obtained five trypanosome strains from raptors (common buzzard, sparrowhawk, kestrel and lesser-spotted eagle), three from passerines (rook, blackbird and finch) and five from blood-sucking insects (blackfly, mosquito, hippoboscids fly and biting midge). Their kinetoplast disc was studied by electron microscopy and the minicircular component of their kinetoplast DNA (kDNA) was analyzed in agarose gels. Selected small subunit (SSU) rRNA genes were PCR-amplified and sequenced using internal primers. In an analysis of trypanosome strains from birds and dipteran insects we have found a clear correlation between the minicircle size and the thickness of the kinetoplast disc. Based on the analysis of the kDNA of flagellates isolated from blackflies *Eusimulium securiforme* and *E. latipes* we conclude that they are very similar to the trypanosomes of raptors, and that these dipterans are their vectors. Furthermore, in the phylogenetic trees based on the SSU rRNA sequences of the trypanosome isolated from *E. securiforme*, this species branched within the "avian trypanosomes" clade. We found three minicircle size categories in four species of birds of prey, and all of them occur in buzzard. Our data suggest that one trypanosome species can develop in different hosts and that one bird species can be infected by several trypanosome species.

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Phospholipase A<sub>2</sub>-like Activity in *Leishmania mexicana*: Participation in the Infection Process. H. ABDALA and P. LE PAPE, Unité de Parasitologie UPRES 1155, Faculté de Pharmacie, Université de Nantes, France.

Some obligate intracellular pathogens use phospholipases-dependent mechanisms for entry and survival inside host. We describe in *Leishmania mexicana* a phospholipase-like activity where the highest rate of hydrolysis (25%) was found in the most infectious stage. The enzyme activity was inhibited by two specific inhibitors of secretory PLA<sub>2</sub> [the p-BPB (10 µM) and the phospholipid analog Decyl-octyl GPC (0.3 µM)] and by the cytoplasmic PLA<sub>2</sub> inhibitor [AACOCF<sub>3</sub> (15 µM)] with values of  $30.1 \pm 2.2$ ,  $87.1 \pm 1.5$  and  $28.1 \pm 2$  %, respectively. The optimal conditions for this global activity (pH 4.5 to 6.0, 37°) corroborates with the microenvironment found in the parasitophorous vacuole of the host mononucleated cells. The PLA<sub>2</sub>-like activity detected in supernatants of promastigotes suspensions was Ca<sup>2+</sup>-dependent, resistant to heating and sensitive to reducing reagent. Further we studied the role of this PLA<sub>2</sub>-like activity inhibition in the invasion process. Parasite treatment before challenge with p-BPB [10 µM] reduced the percentage of infected BALB/c macrophages by  $56.7 \pm 7$  % and the total number of amastigotes per 100 infected and uninfected mac-

rophages by  $51.8 \pm 7.4$  %. Our results suggest that this secretory PLA<sub>2</sub>-like could have an active role in the invasion process. This eventually may be explained by an alteration of the membrane fluidity caused by an arachidonic acid mobilization from macrophage membrane phospholipids.

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Effects of Media on the Lipase Production by *Tetrahymena thermophila*. C. B. PORTOIS,\*\*\*, P. CAILLERET,\*\* I. SADOVSKAYA,\*\* C. DEWEER,\* J. JEANFILS,\*\* and J. DE CONINCK, \*Institut Supérieur d'Agriculture, Lille, \*\*Université du Littoral-Côte d'Opale, Boulogne sur mer, and \*\*\*Faculté Libre des Sciences, Lille, France.

Different compounds were tested in flasks on the whole lipase production (intra- and extra-cellular) by *Tetrahymena thermophila*. Basis medium (MYE) was composed of skimmed milk (1% w/v) and yeast extract (1% w/v). Olive oil or other commercial oils (0.2% v/v), tween 80, 20 (0.16% v/v) or arabic gum (0.2% w/v) were added. Media composed only of yeast extract (w/v) were tested with or without glucose (1% w/v). Fatty acids did not improve *Tetrahymena*'s growth but stimulated the lipase production. Added glucose had the same effect. Different tested oils did not strongly modify growth or lipase production, but with salmon oil, the lipase production was higher and more constantly produced. Emulsifiers improved generation time and final lipase production, but not maximal biomass.

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Macronuclear Chromatin Structure during Resting Excystation of *Colpoda inflata*. M.G. CHESSA,\* L. GALLUS,\*\* L. TIANO,\*\*\* and M.U. DELMONTE CORRADO\*, \*Dipartimento per lo Studio del Territorio e delle sue Risorse, \*\*Dipartimento di Biologia Sperimentale Ambientale ed Applicata, Università di Genova, Genova, and \*\*\*Dipartimento di Biologia Molecolare, Cellulare ed Animale, Università di Camerino, Camerino, Italia.

The macronuclear chromatin structure of *Colpoda inflata* has been studied in situ by image analysis, in order to investigate the activity of regulating mechanisms during resting excystation. Mean histogram on acquired optical microscopy images was calculated in 2- and 25-day-old cysts, coming from a standard culture, as well as in 1-year-old cysts coming from a senescent culture. Moreover, the regions corresponding to the most represented gray levels and ranging from 30% to 50% of the total area of each mean histogram, were identified. Regions so defined were employed for a segmentation of the digital images, in order to identify macronuclear areas similar in their chromatin condensation. This procedure allowed us to evidence the dynamic structure of the macronuclear chromatin. In the highly condensed macronuclear chromatin, the part unable to decondense is extruded as a pycnotic body; afterwards, the remaining chromatin decondenses. The pycnotic feature of the macronuclear extrusion bodies appears similar to that of the degenerating nuclei in animal cells undergoing apoptosis. Supported by the 1999 grant of the University of Genoa.

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Flow Cytometric Comparison of Esterase and Radicals Oxygen Intermediate Productions by *Ostrea edulis* Haemocytes Uninfected and Infected by the Protistan Parasite *Bonamia ostreae*. N. COCHENNEC and S. GARCIA IFREMER, Labora-