THE SIXTH JÍROVEC DAYS

(Bratíkov, May 17–21, 1993)

The XXIII annual meeting of Czech protozoologists, the VIth Jírovec Days (formerly Protozoological Days), organized by the Protozoological Section of the Czech Zoological Society, was held in Bratíkov near Jablonec n. N. in north-eastern Bohemia. Along with the Czech protozoologists, participants from Afghanistan, Poland and Slovakia took part in this meeting. Of the papers presented, eleven abstracts were submitted for publication in Folia Parasitologica. With the exception of two papers they all deal with parasitic protists. These abstracts have not been reviewed and therefore do not represent regular papers and should not be cited as such.

SURFACE LECTINS OF GIARDIA TROPHozoITES

A. C. Majewska and W. Kasprowak

Department of Biology and Medical Parasitology, Karol Marcinkowski University of Medical Sciences, Fredry 10, 61-701 Poznań, Poland

Since mannosyl residues are present on the surface of intestinal epithelial cells we can conclude that lectins may complement the attachment of Giardia to surface epithelium and may contribute to functional and/or morphological damage of small intestine in some individuals with giardiasis. To verify the molecular principle of parasite attachment we studied the presence of specific surface lectin(s) by using Giardia – erythrocyte attachment assay (Farthing et al. 1986). Thirty-two axenically cultured Giardia strains isolated from different species of hosts (Meyer 1970, 1976, Fortess and Meyer 1976, Kasprowak and Majewska 1985, Majewska and Kasprowak 1990) were studied. Specific inhibition of agglutination was performed in the presence of D-Glucose, D-Mannose, N-acetyl-D-glucosamine (GlcNAc), and N-acetyl-neuraminic acid. The results showed that: 1) All Giardia isolates studied had surface lectins with specificities for saccharide residues on experimental rabbit erythrocytes. Since the inhibition of agglutination for particular Giardia isolates differed, we can conclude that there is no unique pattern of surface membrane-associated modality for adherence of Giardia trophozoites to host epithelium by lectin. 2) The highest agglutination activity was observed in isolates from animals. 3) Significant differences in inhibition of agglutination with GlcNAc between human and animal Giardia trophozoites showed that a surface lectin with specificities for GlcNAc might constitute a proper marker for differentiation of both groups of parasites. 4) The Giardia from persons with asymptomatic giardiasis and from patients with clinical giardiasis did not differ in terms of the surface lectin(s). We can also conclude that the transmission of Giardia populations between humans and animals may be a rare phenomenon.

INFLUENCE OF CHRONIC TOXOPLASMOsis ON HUMAN PERSONALITY

J. Flegr and I. Hrdý

Department of Parasitology, Faculty of Sciences, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

The effect of parasitism on host behaviour is being demonstrated in a growing number of host-parasite systems. The induced behavioral patterns often promote transmission of the parasite. The Manipulation Hypothesis suggests that such modification of host behaviour is a sophisticated product of parasite evolution aimed at host manipulation rather than an accidental byproduct of other physiological activities of the parasite. We studied the effect of parasites on host behavior on the Toxoplasma-human model. Three hundred and thirty-eight (338) people were assessed with Cattell’s personality questionnaire and then tested for Toxoplasma gondii infection with a delayed type hypersensitivity test for Toxoplasma. Personality profiles of two groups of subjects, Toxoplasma infected ones (TI) (56 men and 34 women) and Toxoplasma free ones (TF) (139 men and 109 women) were compared using the Hotelling t-test. No difference in personality profiles between TI and TF groups was detected when women and men were considered together (p=0.241) or when only TI and TF women were compared (p=0.204). However, a highly significant difference was detected when the personality profiles of TI and TF men were compared (p=0.025). To determine whether the differences observed were the result of causal relationships between toxoplasmosis and the personality factors or whether it was only a false correlation resulting from the effect of age, a Two-way ANOVA was performed. This analysis showed that for factors G and L (p=0.0032 and 0.0020, respectively) the toxoplasmosis not age was responsible for the observed differences. Discriminant analysis performed on the basis of Cattell’s personality factors enabled differentiation of the group of subjects suspected of having toxoplasmosis. In the group of 14 subjects classified as Toxoplasma positive on the basis of the factors G and L, the frequency of toxoplasmosis was 64% (a priori probability, the frequency in the unsorted population was 28.7%). When factors F and Q2 were included into classification function, the frequency of correctly diagnosed toxoplasmosis increased up to 73.7%.
WESTERN BLOT ANALYSIS OF *TOXOPLASMA GONDII* CIRCULATING ANTIGENS

P. Kodym

National Institute of Public Health, Šrobárova 48, 100 42 Praha 10, Czech Republic

Circulating antigens can be found in blood sera of approximately 5% of patients with acute, chronic or latent toxoplasmosis. The aim of the present work was to determine, which antigenic components of *Toxoplasma* circulate in human bloodstream.

Human sera, in which circulating antigens were detected by DOT ELISA, were run on a 12.5% SDS-PAGE and electrophoretically transferred to nitrocellulose sheets. The antigens were recognized by rabbit anti-*T. gondii* antibodies and visualized using streptavidin-biotin system (BIORAD). This procedure revealed 5 conspicuous and 3 additional less visible bands, corresponding to major *Toxoplasma* antigens (virulent P strain) which ran in parallel. The most prominent was the band of approximate relative molecular weight of 57 kDa, followed by the bands at 50 kDa, 32 kDa, 30 kDa and 28 kDa. Very weak were the bands of relative molecular weights 115 kDa, 100 kDa and 24 kDa. According to literature, the 57 kDa and 50 kDa bands represent rhoptry antigens, whilst the antigens at 32 kDa, 30 kDa and 28 kDa are of membrane origin.

TWO-STEP FREEZING OF THE RUMEN CILIATE PROTOZOOON *OPHYROSCOLEX CAUDATUS TRICORONATUS* AND LONG-TERM PRESERVATION IN LIQUID NITROGEN

S. Kišidayová

Institute of Animal Physiology, Slovak Academy of Sciences, Palackého 12, 040 01 Košice, Slovakia

The freezing procedure of the rumen protozoon *Ophryoscolex caudatus tricoronatus* is described. The best average survival achieved after 24 hours storage in liquid nitrogen (LN2) was 30% under these conditions: 15 min equilibration with 4% DMSO (dimethylsulphoxide) as a cryoprotectant at 30°C and 45 minutes holding phase at the holding intermediate temperature −40°C followed by immersion of the protozoal suspension into LN2. The protozoal concentration in the cryopreserved suspension was 1000–2000 x ml⁻¹ and the cooling rate was was around 1–1.8°C min⁻¹. The growth ability was confirmed by cultivation in vitro. No changes in the recovery of protozoa after 3 months of cryopreservation was observed. The small decrease of the protozoal recovery was observed after 2 years, but without effect on growth ability. This work is presenting the first successful cryopreservation of the rumen protozoa *O. caudatus tricoronatus*.

KARYOTYPE OF *TRICHOMONAS VAGINALIS*

T. Drmota and J. Král

Department of Parasitology and Department of Zoology, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

Chromosomes of *Trichomonas vaginalis* were studied on slides prepared by the spreading technique. The trichomonad culture was incubated to a concentration 10⁶ cells/ml. After colchicine treatment incubation was continued after 6 hours. Colchicine arrested cells at metaphase. Cells were hypotonized by 0.075M KCl and placed into Carnoy fixative. The final suspension was dropped on to icecd slides and stained with Giemsa solution (5% Giemsa in Sörensen buffer, pH=7.0; 5 minutes).

The *Trichomonas vaginalis* karyotype is haploid and consists of six different chromosomes ranging from approximately 2.40 to 1.00 µm long (Table 1). The typical karyotype contains 1m-1m/sm-1sm/st-st/1a-2a/t chromosomes. Occasionally diploid metaphases and stages with four nuclei were observed both on colchicine treated and untreated slides. We speculate that these stages constitute phases of meiosis. The presence of the diploid metaphases implies the existence of a diploid stage in the life cycle of *Trichomonas vaginalis* which, however, represents very small fraction of the cultured population.

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>Relative size (%)</th>
<th>Arm ration</th>
<th>Nomenclature*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>100.0</td>
<td>3.60 ± 0.90</td>
<td>sm/st</td>
</tr>
<tr>
<td>II</td>
<td>75.4 ± 6.0</td>
<td>∞</td>
<td>a/t</td>
</tr>
<tr>
<td>III</td>
<td>67.3 ± 5.4</td>
<td>1.22 ± 0.12</td>
<td>m</td>
</tr>
<tr>
<td>IV</td>
<td>58.8 ± 7.4</td>
<td>1.56 ± 0.37</td>
<td>m/sm</td>
</tr>
<tr>
<td>V</td>
<td>50.7 ± 5.8</td>
<td>no*</td>
<td>st/a</td>
</tr>
<tr>
<td>VI</td>
<td>42.3 ± 6.0</td>
<td>∞</td>
<td>a/t</td>
</tr>
</tbody>
</table>

* m-metacentric, sm-submetacentric, st-subtelocentric, a-acrocentric, t-telocentric
* Short arm was not measured
SUPEROXIDE DISMUTASE ACTIVITY IN TRICHOMonas VAGINALIS

E. Tomková and J. Tachezy

Department of Parasitology, Faculty of Sciences, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

Superoxide dismutase (SOD) protects organisms against the toxic action of superoxide formed in various biological oxidations under aerobicosis. Its activity was observed in the majority of aerotolerant cells including facultative anaerobe Trichomonas vaginalis. In our experiments we attempted to find whether or not the trichomonads exposed to oxidative stress increase the activity of SOD to reinforce their anti-oxidant defense system.

The trichomonads were incubated for 12 hours in Diamond’s TYM medium, continually equilibrated with atmosphere containing 5% O₂ and 95% (95% N₂ + 5% CO₂). Control cells were incubated under the same conditions without oxygen. The activity of SOD was determined in dialyzed cell homogenate using xanthine-xanthine oxidase-cytochrome c assay. The results showed that SOD activity increased about 10-fold (10 U/mg) in trichomonads incubated in the presence of 5% O₂ in comparison to those incubated under anaerobiosis (1 U/mg).

It has been reported that metronidazole-resistant strains of T. vaginalis have an impaired oxygen scavenging system which causes their lowered tolerance to oxygen. Therefore we investigated whether there are any differences in the activities of SOD between the drug susceptible and resistant strains. We compared SOD activities of the parent TV 10-02 strain with those of its resistant derivatives able to grow at 3 (MR-3) and 5 µg/ml metronidazole (MR-5) under anaerobiosis. This study revealed 3.9-fold (3.9 U/mg) and 5.5-fold (5.5 U/mg) higher activity of SOD in resistant derivatives MR-3 and MR-5, respectively, in comparison to the parent drug susceptible strain (1.0 U/mg).

Oxygen dependent induction of SOD activity in T. vaginalis apparently represents parasite adaptation to oxidant challenge. Higher production of H₂O₂ by increased SOD activity, however, needs to be accompanied by adjustment of other antioxidant mechanisms. It is apparent that adjustment of antioxidant enzymes is distorted in drug-resistant T. vaginalis, which are more sensitive to oxygen in spite of higher SOD activities. However, mechanisms detoxifying H₂O₂ in T. vaginalis, which lack both catalase and peroxidase, are not known.

SEROLOGICAL RESPONSE AGAINST POULTRY COCCIDIA AFTER VACCINATION BY LIVA-COX T, EVALUATION BY ELISA AND IFAT

M. Provazníková, A. Firmanová and P. Bednák

Research Institute of Feed Supplements and Veterinary Drugs, 254 49 Jihlava near Prague, Czech Republic

Seven-day-old chickens were immunized with doses of either 500 or 5000 oocysts of attenuated lines of Eimeria tenella, E. acervulina and E. maxima (LIVACOX T). The oocysts were administered via drinking water or directly into the crop. Sera from infected chickens were collected between 5 and 56 days after immunization (DAI), and tested by IFAT and ELISA. Sporozoites of E. tenella were used as antigen for IFAT and saline extract of broken sporulated oocysts of E. tenella, E. acervulina and E. maxima for ELISA.

Low levels of antibodies were detected in sera collected 5 DAI examined by IFAT. The highest titres were found 14 DAI (1:1280). Titres between 1:300–400 (average of 20 sera) were still observed 41 DAI. No marked differences in serological response after various immunizations were found by ELISA. The highest levels of IgG antibodies were found 5 DAI. These antibodies were probably of maternal origin. Many cross reactions were observed between species of Eimeria in ELISA. This indicates the existence of common antigens in oocysts and sporocysts of different species. Soluble oocyst’s antigen is not suitable for determination of immunological response against various species of poultry Eimeria by ELISA.

A NEW DIAGNOSTIC METHOD FOR THE PROTEOLYTIC ACTIVITY DETERMINATION OF THE RUMEN PROTOZOOON ENDOTINUM CAUDATUM USING DOT-BLOT TECHNIQUE

A. Marcin, *S. Kišidaková, E. Bellčíková and P. Siklenka

Institute of Experimental Veterinary Medicine, Košice, Slovakia; *Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovakia

The purpose of this study was to develop a method suitable for the diagnostic of specific proteolytic activity of the rumen protozoon Entodinium caudatum. As a substrate were used wheat proteins (albumine and globuline fraction isolated from the wheat seed). The concentration of the protozoa in the culture medium was 10,000–20,000 cells/ml. The samples were taken at 2 h intervals and they were used for the determination of the total protein concentration (measured by Bradford method), wheat protein concentration in the medium (measured by both methods mentioned above) and nonspecific proteolytic activity (asocasein-substrate). Degradation rate varied between 0.027–0.073 mg/ml per min and nonspecific activity 0.003–0.007 mg/ml per min. This method provides a suitable model for the quantification of the proteolytic activity of the rumen protozoon E. caudatum during the experiments in vitro.
CHANGES IN THE INTERNAL ULTRASTRUCTURE OF MICROSPORIDIA SPORES DURING LONG-TERM STORAGE
Z. Žižka and Z. Hostounský
Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Praha 4; Palouky 608, 253 01 Hostivice, Czech Republic

To obtain a successful experimental infection by microsporidia it is necessary to observe the following conditions: (1) the spores should be live and capable of releasing the germ inside a suitable host; (2) there should be a sufficient number of germs to overcome the defence mechanisms of the host. Spores of Nosema sp. from beetles Otiorrhynchus morio were bred using a laboratory host Gastrophysa viridula fed on Rumex obtusifolius (Hostounský 1984). A spore suspension was prepared from images using the method of Hostounský (1981). The spores were stored for 12 months at room temperature and for 2 months in a refrigerator at 4–8°C. The material was fixed in 4% glutaraldehyde, postfixed in 1% OsO4 and embedded in Eupal W. Ultrathin sections were examined in a JEOL JEM 100 B electron microscope. The entirety of spore ultrastructure served as a criterion of their quality. The spore mixture contained 56% of mature spores and 44% of sporoblasts, which were strongly deformed by fixation. According to our observations in the electron microscope, only 6–7% spores were relatively well preserved and probably capable of infection even after a long-term storage. During storage the postosome was destroyed first, the germ with two nuclei was damaged and, eventually, alterations of polaroplast (the lamellar part lasted longer than the vesicular one) and polar filament occurred. The least damaged part of the spore was its envelope (thick osmiophilic exospore and a 50% thicker electron-lucid endospore). In all Nosema spores, even in those which were the least preserved, several coils of the polar filament and remnants of the polaroplast could be distinguished beside the envelope layers, if the internal part of the spores was not lost during the preparation, e.g. when ultrathin sections were cut.

DYSFUNCTIONAL STATES OF THE CYTOSKELETON
R. Janisch
Department of Biology, Faculty of Medicine, Masaryk University, João de 10, 66244 Brno, Czech Republic

The cytoskeleton has three basic roles in the cell: it is responsible for the structure, movement and epigenic information of the cell. Impairment of any cytoskeletal component, i.e., microtubules, microfilaments or intermediary filaments, results in defects of that function for which the respective component is responsible. There are two types of causal relationships between an impaired cytoskeletal function and a pathological state of the cytoskeleton: 1) a defect in the cytoskeletal structure is the cause of pathological manifestations; 2) a pathological situation produces defects in the cytoskeleton, which has further consequences. In both categories, many examples of defects in microtubules and microfilaments are known.

The first category includes microtubular disorders shown by impaired ciliary movement. Errors in the structure of ciliary axonema or kinetosomal microtubules or changes in links between microtubules and associated proteins subsequently interfere with metachronal rhythm and produce loss of coordination of ciliary movement. Excessive multiplication and redistribution of microtubules disturbs transmission of chemotactic signals from the membrane, which affects cell locomotion. In multicellular organisms, these changes may produce a whole range of diseases, such as Kartagener’s syndrome. Abnormal actin or impaired polymerization of actin into microfilaments may cause a decrease in leucocyte motility, known as the "lazy leucocyte" syndrome.

The second category covers neurodegenerative disorders, muscle atrophy, changes associated with aging of cells or malignant transformation of cells. Oncogenes are also closely related to the cytoskeleton; some of their products may interfere with the synthesis of cytoskeleton proteins.

An understanding of mechanisms underlying dysfunctions of the cytoskeleton, based on studies of their causes and consequences, is a prerequisite for control of these processes and their possible rectification.

DECREASE OF MALATE METABOLISM IN HYDROGENOSOMES OF TRICHOMONAS VAGINALIS DURING DEVELOPMENT OF METRONIDAZOLE RESISTANCE
J. Tachezy, E. Tomkova, T. Drmota and J. Kulda
Department of Parasitology, Faculty of Sciences, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

Cytotoxic action of metronidazole on trichomonads depends on ferredoxin-mediated reduction of the drug in hydrogenosomes. It has been found that electrons employed in this process are generated by activity of the hydrogenosomal enzyme pyruvate: ferredoxin oxidoreductase (PFO). Decrease and eventual loss of this activity results in the anaerobic drug resistance in Tritrichomonas foetus. In Trichomonas vaginalis, however, the PFO activity is lost at an early stage of resistance development (MLC=15.7 µg/ml; MR=5) as evident from our examination of metronidazole resistant derivatives growing in vitro at 3, 5 and 100 µg/ml metronidazole (MR-3, MR-5 and MR-100, respectively). These findings suggest the presence of an alternative source
of electrons, which has to be eliminated before full drug resistance is achieved. As it is known that NADH can also provide electrons for metronidazole reduction we followed the activities of NAD : ferredoxin oxidoreductase and malate dehydrogenase decarboxylating (malic enzyme) in T. vaginalis strains at different levels of resistance development. We found that the development of resistance is accompanied by a 75-fold decrease of activity of NAD : ferredoxin oxidoreductase in the large granules fraction (LGF) of the parasite (from 60329 nmol/min/mg in the parent strain to 8.249 nmol/min/mg in MR-100). A moderate decrease in this activity also was observed in derivatives MR-3 (342167 mol/min/mg) and MR-5 (25173 nmol/min/mg). Stepwise development of resistance was further accompanied by gradual decrease and final loss of the activity of malic enzyme (1876689; 564113; 433129 and 0 nmol/min/mg in the parent, MR-3, MR-5 and MR-100 strains, respectively). To compare expression of protein corresponding to malic enzyme activity, LGFs of examined strains were also analyzed by SDS-PAGE. In fractions isolated from the parent strain and resistant derivatives MR-3 and MR-5 malic enzyme was apparent on electrophoretic gels as a major protein of 59 kDa. In a derivative with fully developed resistance (MR-100) the corresponding band was at the limit of detectability, indicating a marked decrease in expression of malic enzyme in these organisms.

Presented results suggest that activities of NAD : ferredoxin oxidoreductase and malic enzyme participate in reductive activation of metronidazole in hydrogenosomes of T. vaginalis. The development of full anaerobic resistance to metronidazole in this species therefore requires elimination of this system in addition to inactivation of the PFO dependent drug activation process.