MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

Effect of Cyclosporine A on *Leishmania tropica*. Nancy C. Behforouz, Department of Biology, Ball State University, Muncie, Indiana 47306.——The effect of Cyclosporine A, a new immunosuppressive and antiparasitic drug was tested, both *in vivo* and *in vitro*, on *Leishmania tropica*. *In vitro*, the drug inhibited growth of the parasite and decreased the infectivity of the organism. Although this drug appeared to have little or no therapeutic effect for susceptible, infected mice at the doses tested, it had a significant, dose-dependent prophylactic effect when used two days prior and five days following infection.

The Regulation of S-Adenosylmethionine Synthetase in *Candida albicans*. Richard H. Lambert, Eli Lilly and Company, Indianapolis, Indiana 46285 and J.R. Garcia, Ball State University, Muncie, Indiana 47306.——S-Adenosylmethionine (SAM) synthetase from yeast and hyphal-phase cells of the dimorphic fungus *C. albicans* was characterized by kinetic analysis and response to inhibitors. SAM Synthetase is the enzyme responsible for the synthesis of S-Adenosylmethionine (SAM), the compound which serves as the major methyl-group donor in the methylation of macromolecules such as DNA, RNA, and proteins. The enzyme from yeast-phase cells has a km of 0.17 mM for methionine, 0.14 mM for ATP, and is inhibited (*in vitro*) by dimethylsulfoxide, methionine sulfone and methionine sulfoxide. They hyphal-phase SAM synthetase has a km of 0.056 mM for methionine, 0.02 mM for ATP, and its activity (*in vitro*) is enhanced by the inhibitors used with the yeast-phase enzyme. This preliminary data strongly suggests that isozymes of SAM Synthetase are present in *C. albicans* and possibly that the isozymes are morphology-specific.

The *in vivo* studies revealed that the enzyme's synthesis is repressed by the addition of methionine and that the specific activity increases during a temperature-induced shift in morphology. In addition, it was shown that the increase in specific activity (seen during a yeast → hyphae shift and/or when yeast cells, grown in a methionine-supplemented medium, are transferred to a methionine-free medium) involves *de novo* protein synthesis.

A Case of Tuberculosis in the University Setting. M. Langona, Department of Epidemiology, Ball Memorial Hospital, Muncie, Indiana 47303.——Since the 1970s the United States Public Health Service has worked extremely hard in preventing the

transmission of communicable and infectious diseases within this country by Asian refugees. Mandatory health screening tests for tuberculosis, leprosy, venereal disease and other medical conditions have been provided while the refugee is still abroad, and then again upon arrival at various U.S. ports of entry. Unfortunately, Asians who are not refugees may immigrate into this country without appropriate health testing and may represent a public health problem.

This presentation will describe a case of pulmonary and extrapulmonary tuberculosis diagnosed in a young, pregnant Korean who recently arrived in Indiana with her spouse who is a foreign-exchange university student. Unfortunately, the university's health policy only required tuberculosis skin testing of the enrolled student and not the spouse. Information will be provided about the diagnosis, epidemiologic workup, hospitalization of the tuberculosis patient, and the dichotomy of the public health regulations.

Scabies: A Nosocomial Outbreak. M. LANGONA, S. BOSSUNG, AND M. ORR, Department of Epidemiology, Ball Memorial Hospital, Muncie, Indiana 47303.——*Sarcoptes scabiei* (var. hominis) an obligate ectoparasitic mite of humans continues to present itself as a health problem within the United States. Although scabies is a non-reportable disease and reliable data on its incidence is limited, several investigators as well as the Centers for Disease Control report that the United States is experiencing the most significant increase in scabietic infestations since the epidemics of World War II.

This presentation will describe a 1984 epidemic of Norwegian (crusted) Scabies which involved the admission of a nursing home patient into a community-teaching hospital and the subsequent nosocomial scabies outbreak of 15 hospital personnel and their families.

The suspicion of scabies with supportive clinical and laboratory findings warrants control measures, and dependent on the form of scabies present, the immediate and efficacious epidemiologic investigation within the hospital setting.

Although the 20th century clinician possesses a simple and effective cure for scabies infestations, it is indeed disheartening that we lack the ability to eradicate this nuisance mite.

Three Plasmid Cloning Vectors for Mammalian Cells. STEVEN H. LARSEN AND JOANN HOSKINS, Department of Microbiology and Immunology, School of Medicine, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Plasmid vectors based upon selection of the dominant phenotype of resistance to the G418 antibiotic have been developed. To provide this resistance, the coding sequence for the Tn5-derived aminoglycoside phosphotransferase activity were sandwiched between the promotor and polyadenylation signals of the thymidine kinase gene from herpes simplex virus. This construct was placed into ampicillin or ampicillin-tetracycline resistant derivatives of pBR322. One such construct, pSL72, can be stably selected in mouse L293 cells at an efficiency equal to any previously known system (greater than 0.1% of the cells). This plasmid appears to be selectable at a single copy per ceil. A second vector, pSL71, is quite similar except that the copy number can be increased to about 100 genomes per animal cell. The third plasmid includes mouse cell DNA sequences which provide the plasmid with the ability to be maintained extrachromosomally and hence recovered again from the animal cell into bacteria.

Banking DNA for Future Diagnosis of Hereditary Diseases. LINDA MADISEN AND M.E. HODES, Indiana University School of Medicine, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Recombinant DNA methodology

is becoming increasingly important for the detection of the carrier state of a number of genetic diseases. After generating a series of restriction fragment length polymorphisms closely linked to a gene causing a disease, it is possible to predict whether an individual has inherited the haplotype associated with the deleterious gene. Such studies require DNA from informative relatives as well as from affecteds and so will require the long term storage of highly polymerized DNA, a relatively new procedure whose limitations are still being investigated.

By storing DNA at temperatures above 4°, one may cause accelerated aging and thus mimic long term storage. We found that DNA stored in solution at −70°, −20°, 4°, 25° and 37°C for two months remains high molecular weight. Early results indicate these different storage temperatures have no effect on restriction enzyme banding patterns for Xbal, HindIII and EcoRI. Similar incubation of the DNA at 65°C resulted in extreme degradation. Furthermore, blood stored at −70°C for two months prior to extraction generally yielded a quantity of high molecular weight DNA comparable to fresh samples. Occasional frozen samples, however, yielded considerably lower DNA quantities, all of which were high molecular weight.

An Examination of 495 Splice Junction Sequences. F.H. NORRIS, Eli Lilly and Company, Indianapolis, Indiana 46285, and M.E. HODES, Indiana University School of Medicine, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——We have performed a computer aided examination of 495 of the exon-intron junctions reported in the June, 1984, Genetic Sequences Databank (GenBank). We examined the junction data as a pool and also segregated according to organisms in which they occur. The consensus sequence we found, 5'-(AC)AG/GT(AG)AGT, is the same as that reported by Mount and others. We also find that, except for the A or G at position +3, conservation of the sequence is highest near the splice point and drifts with distance, with the bases on the intron side of the junction being more highly conserved. The nine bases indicated by the consensus sequence seem to define the junction, since we find no conserved bases within 60 bases of the splice site. Beyond the −3 and +6 boundaries, the bases are randomly distributed.

The frequencies of occurrence of the junction sequences were tabulated. We find no differences between the human-ape sequence frequencies and the frequencies of the other mammalian sequences. Striking differences appear as one compares sequences from higher and lower organisms. Over 60% of the human, but less than 40% of the non-mammalian vertebrate sequences, are of the form /GT(AG)AG. More than a third of the lower vertebrate junction sequences occurred one time. Perhaps because of a host-virus relationship, we see fewer differences between human and viral than between viral and lower vertebrate sequences.

Transcriptional Regulation of the Sporulation-specific Glucoamylase of *Saccharomyces cerevisiae*. TOM PUGH AND MARY CLANCY, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556.——Sporulating cells of the yeast, *Saccharomyces cerevisiae* contain a glucoamylase activity (SAG) which is distinct from similar enzymes found in vegetative cells. The enzyme is a glycoprotein and is capable of releasing free glucose from maltotriose, maltodextins, amylose and glycogen, but maltose is hydrolyzed slowly, if at all. The time of appearance of SAG activity during sporulation corresponds to the onset of glycogen degradation, and immediately proceeds spore formation.

We have been interested in differential gene expression in sporulating yeast and would like to know the level at which regulation of SAG activity occurs. SAG expression is prevented if cycloheximide is added to sporulating cells at any time before full

levels are attained. Antibody prepared against 400-fold purified enzyme specifically precipitates a protein of 68K daltous from extracts of sporulating cells which have been pulse-labelled *in vivo* ^{35}S-methionine. This band is not detected at early times in sporulation or in non-sporulating cells. This shows that the regulation of SAG activity is not post-translational and suggests that control may be transcriptional.

We have constructed a library of *S. cerevisiae* DNA in the expression vector, pBD6, and are screening for the SAG gene, using a plate assay and antibody techniques.

Development of a Model System for the Study of Murine Leukocyte Chemiluminescence. JAMES L. SHELLHAAS, Butler University, Indianapolis, Indiana 46208.——A model system was developed for the determination of the activation kinetics of murine peripheral blood polymorphonuclear neutrophils (PMN's). Utilizing discontinous density gradient centrifugation and dextran sedimentation, populations of PMN's were prepared of 98% purity. These cell populations were then examined for their ability to respond with luminol-dependent chemiluminescence upon co-cultivation with the chemotactic peptide N-formylmethionine-leucine-phenylalanine (Fmet), the tumor promotor phorbol myristic acetate (PMA), and opsonized zymosan. Purified populations of PMN's were also examined for their responsiveness in chemotactic assays to each of the stimulation agents. Significant differences between the chemiluminescent kinetics of murine cells and the published kinetics of human cells were observed. Chemotactic responsiveness also differed in murine cells from that observed in human PMN cell populations.

Relationship between Symptomatic Resistance and Virus Production in Barley Cultivars Inoculated with Barley Yellow Dwarf Virus. M. SKARIA, J.E. FOSTER AND R.M. LISTER. Departments of Botany and Plant Pathology and the U.S. Department of Agriculture (Foster, Purdue University, West Lafayette, Indiana 47907.——Resistance to barley yellow dwarf virus (BYDV) disease has been identified in some Ethiopian barleys. A genetic factor, the "Yd_2" gene associated with symptomatic resistance has been transferred to several barley cultivars. Few such barleys are available as near-isogenic pair with the only difference in presence or absence of the Yd_2 gene. We investigated the effect of the Yd_2 gene on virus synthesis in three near-isogenic barley pairs. One week old plants of California Mariout (Yd_2-) barley and the near-isogenic CM 67 (YD_2+) were inoculated with PAV, MAV, or RPV isolates of BYDV (i.e. transmitted by *Rhopalosiphum padi* L. and *Sitibion avenae* (Fabr.; by *S. avenae*; or by *R padi*, respectively). Inoculated plants were grown in a growth chamber at 20 ± 1°C. The virus content of shoots and roots was assessed at six day intervals for one month by enzyme-linked immunosorbent assay (ELISA). With PAV, overall significantly less virus was detected in CM 67 than in California Mariout, but with MAV and RPV there were no such differences. In other experiments PAV production behaved similarly in Prato (Yd_2+) barley and the near-isogenic Briggs (Yd_2-), and in Atlas 68 (Yd_2+) barley and the near isogenic Atlas 57 (Yd_2-). Thus, symptomatic resistance to BYDV in barley correlates with reduced virus synthesis.

Serum Hormone Levels in Germfree and Conventional Rats: Effect of Dietary Restriction. DAVID L. SNYDER AND BERNARD S. WOSTMANN, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——Germfree* rats were used to obtain background information on the relationship between aging, hormone levels, and restricted dietary intake. Blood samples were obtained by heart puncture from 14 conventional

*_Actinomyces_ sp. had previously contaminated the isolators of these GF rats. However, fecal smears did not indicate growth of these organisms in the intestinal tract.

(CV), 27 germfree (GF), and 12 germfree but restricted (GR) Lobund-Wistar rats. Intake for the restricted rats was 70% of *ad lib.* intake. All rats were males, 8 to 12 months old, and fed natural ingredient diet L485. Samples were collected between 10 A.M. and 12 P.M., under halothane anesthesia, and after an overnight fast. GF rats had slightly lower serum insulin than CV rats (52.9 vs. 62.6 uU/ml) but GR were significantly ($P < 0.01$) lower than GF (52.9 vs. 35.2 uU/ml). Serum glucose levels paralleled insulin levels (CV:140; GF:114; GR:98 mg/dl). No significant differences were found in total thyroxine (T4) levels (CV:6.2; GF:5.5; GR:5.5 ug/dl) and in total triiodothyronine (T3) levels (CV:115; GF:134; GR:133 ng/dl). Significant differences were found among the testosterone (T) levels. GR rats had higher ($P < .02$) T levels than Cv rats (3.4 vs. 2.1 ng/ml). GR rats had higher ($P < .01$) T levels than GF rats (7.8 vs. 3.4 ng/ml). The reduction in insulin levels in GR rats may be a response to lower caloric intake and an effort to maintain glucose levels through gluconeogenesis. Other possible factors affecting insulin and glucose levels are the lower metabolic rate of GF animals, changes in thyroid hormones, and the anabolic effects of testosterone. Though body weights of GR rats were only 72% of GF rats, GR rats maintained testes sizes similar to GF rats. However, this could account only in part for the higher serum T concentrations of GR rats. Our findings suggest that dietary restriction of even 30% is enough to modify hormone patterns which in turn may lead to the extended lifespan observed in these animals.