

MICROBIOLOGY AND MOLECULAR BIOLOGY

Chairman: DAVID C. MADSEN, Lobund Laboratory
University of Notre Dame, Notre Dame, Indiana 46556

RALPH L. NICHOLSON, Department of Botany and Plant Pathology
Purdue University, Lafayette, Indiana 47907
was elected Chairman for 1976

ABSTRACTS

Callus and Cell Suspension Cultures of Maize. T. H. OSWALD and R. L. NICHOLSON, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974). A modified Linsmaier and Skoog medium was used for induction of callus on mesocotyls and roots of maize seedlings. Amendments with vitamins and an increased concentration of chelated iron accelerated callus induction 3 fold, compared with the rate of callus induction on the medium lacking these modifications. Continued growth of excised callus, through repeated subcultures, was possible by lowering the concentration of sugar and adjusting the balance of mineral nutrients, in addition to maintaining the increased concentration of chelated iron and providing the vitamin supplements. Cell suspension cultures were established from callus placed in liquid medium in which the chelating agent was omitted. This liquid preparation contained non-chelated iron, acetate and the aforementioned modifications.

Comparison of Growth Rate and Mycelial Density of *Rhizoctonia Solani* on an Ammoniacal and Nitrate Source of Inorganic Nitrogen. CYNTHIA M. KUIVENHOVEN, D. M. HUBER, and H. L. WARREN, Department of Botany and Plant Pathology, and ARS-USDA, Purdue University, West Lafayette, Indiana 47907.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974.) The form of nitrogen fertilizer used greatly influences the severity of plant diseases caused by *R. solani*. Nitrate nitrogen ($\text{NO}_3\text{-N}$) reduces stem canker of potato and other *Rhizoctonia* diseases, while Ammonical nitrogen ($\text{NH}_4\text{-N}$) may increase severity. To determine if the form of nitrogen exerted a direct effect on growth of the pathogen, various isolates of *R. solani* from several hosts were grown on cellophane membranes overlying a modified Czapek-Dox agar medium containing either $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ as the nitrogen source. Although differences in lineal growth were observed between isolates, there were no differences in growth rate attributable to nitrogen source. Mycelial density was also similar for the two forms of nitrogen when the medium was strongly buffered to prevent a low pH with $\text{NH}_4\text{-N}$. Pectolytic and cellulolytic enzymes were produced with $\text{NH}_4\text{-N}$ but not with $\text{NO}_3\text{-N}$. This data suggests the form of nitrogen influences *Rhizoctonia* diseases by affecting pathogenicity rather than growth of the pathogen.

NMR Characterization of ADP-ATP Translocase. WILLIAM S. KAIN, Indiana State University, and WALTER X. BALCAVAGE, Center for Medical Education, Indiana University School of Medicine, at Indiana State University.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974.) The NMR spectra of ADP-nucleotide translocase complexes show enhanced spin relaxation of H_8 on the purine ring in the presence of mitochondrial ghosts. This effect is atractyloside sensitive. Atractyloside added to the complex induced further H_8 peak broadening at half maximal concentration of $4 \mu\text{m}$ with 20 mg/ml membrane protein. Atractyloside added before ADP eliminates the specific H_8 peak broadening. No spin relaxation effects are seen at the H_2 proton. Assuming a binding site concentration of 600 p moles per mg protein, the lifetime of the ADP-translocase complex can be calculated to be approximately 20μ sec. Similar calculations show the lifetime of the ADP-Translocase complex in the presence of atractyloside to be about 46μ sec. EDTA competes with ADP for binding to the translocase, reversing the NMR peak broadening and EDTA inhibits state 4 to state 3 respiratory transitions. A lipid free aqueous suspension of mitochondrial membrane proteins causes effects on the NMR spectrum of ADP identical to those described above. The spin relaxation phenomena shown here are consistent with a model of the translocase containing a paramagnetic metal, at or near the active site.

Induction of Resistance in Cucumbers and Recognition of the Presence of a Compound Fungitoxic to *Cladosporium Cucumerinum*. G. S. ACRES, R. E. HAMMERSCHMIDT, and J. KUC, Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974.) Eight-day-old cucumber (*Cucumis sativus* L.) seedlings, resistant or susceptible to scab and all susceptible to anthracnose, were inoculated with *Colletotrichum lindemuthianum* (Sacc. and Magn.) Schribner beta race, bean anthracnose causal agent, *Colletotrichum lagenerium* (Pass.) Ell. and Halst. race 1, causal agent of cucurbit anthracnose, or *Cladosporium cucumerinum* Ell. and Arth., cucumber scab causal agent. Scab susceptible plants were found to have resistance to scab induced when treated with *C. lindemuthianum* 24 hours prior to a challenge with *C. cucumerinum*. Plants susceptible to anthracnose, but resistant to scab, had resistance to anthracnose induced by treatment with *C. cucumerinum* 24 hours prior to a challenge with *C. cucumerinum*. Plants susceptible to anthracnose, but resistant to scab, had resistance to anthracnose induced by treatment with *C. cucumerinum* 24 hours prior to a challenge with *C. lagenerium*. Water extracts of etiolated seedlings of two genetically similar varieties, except with respect to scab resistance, were dialyzed against water, and the membrane permeable fraction saved. Two compounds were found by paper chromatography that fluoresced under UV light and reacted with diazotized sulfonilic acid. UV quantitation showed the same high level of the compounds in scab infected and healthy resistant tissue. Less was found in healthy susceptible tissue and the least in infected susceptible tissue. An on chromatogram inhibition assay, OCIA, showed that one of the spots inhibited the growth and germination of *C. cucumerinum*.

Preferential Assignment of Allotype al Globulin for Production of Early IgM anti-p-azophenylarsonate Antibody in al, a3 Heterozygous Rabbits. C. C. CHIEN and J. S. INGRAHAM, Department of Microbiology, Indiana University School of Medicine, Indianapolis, Indiana 46202.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974.) It has been observed that in some rabbits with immunoglobulin of allotype al, a3 the IgG anti-arsanilazo antibody is chiefly or perhaps exclusively of allotype al. (Zimmerman et al., *Fed. Proc.* 32, 1014 (1973). For the present study, a group of seven rabbits with immunoglobulin of allotype al, a3 was immunized with a mixture of p-azophenylarsonate (AA) pigeon red cell stromata and p-azophenyl-N-trimethylammonium (TMA) pigeon red cell stromata intravenously on days 0, 2, and 4, and blood lymphocytes were isolated by the Ficoll-Hypaque technique on day 6. Two of the animals were autopsied on day 6 and spleen cells were also examined. The proportion of the antibody-forming cells in these lymphocytes which were making IgM anti-AA antibody of allotype al or a3 was estimated by inhibition of hemolytic plaque formation on AA-sheep RBC by means of anti-allotype antisera. In seven animals of allotype al, a3 the number of anti-AA plaque-forming cells (PFC) was inhibited 71% (45-99%) by an anti-al antiserum and only 13% (1-30%) by an anti-a3 antiserum. In the same animals, the number of anti-TMA PFC was not inhibited by the same anti-al antiserum, except for spleen cells from one animal which were inhibited by 30%. However, inhibition of the TMA PFC by the anti-a3 antiserum was 34% (1-69%). Thus, it appears that the great majority of the anti-AA PFC were making antibody of allotype al. Subsequent study of specific inhibition of the anti-AA hemolytic activity in the sera of some of these animals also showed the activity to be carried chiefly by molecules of allotype al. On the contrary, by the same criteria, the activity of anti-TMA appears to be higher in the a3 than in the al. It seems of interest to inquire whether this preferential selection of one allotype of globulin over the other in anti-AA immune response is simply a fortuitous aspect of the genetics of these animals or if it reflects a significant aspect of the structural properties of the allotypic globulins in relationship to certain antigens.

Factors Affecting Bacterial and Fungal Growth in Total Parenteral Nutrition Solutions. M. L. FAILLA, D. D. BENEDICT,* and E. D. WEINBERG, Department of Microbiology, Indiana University, Bloomington, Indiana 47401.—(Presented at 1974 meeting, erroneously omitted from *Proceedings* of 1974.) Growth of starved bacteria and yeasts in the components of and in complete parenteral nutrition solutions (TPN) prepared with 8.5% Freamine, 5% Aminosol, or 10% vitamin-free Casamino acids was examined. Yeasts grew well in all TPN solutions employed, whereas bactericidal effect observed in TPN-Aminosol was overcome by raising its pH of 5.1 to 6.1 (the natural pH TPN-Freamine). The bactericidal nature of TPN-Freamine was altered neither by increasing the pH to neutrality nor by decreasing its osmolality. However, when Freamine was diluted threefold or greater prior to its incorporation in the TPN solution, bacteria were able to grow rapidly; this suggests

*Represents author presenting paper.

that the bactericidal activity may result from the concentration of certain amino acids or other factors inherent in the Freamine solution. The effects on growth of supplementation of TPN solutions with metals, vitamins and inorganic phosphate (Pi) were also observed. In diluted TPN solutions, exogenous Pi greatly enhanced bacterial growth. Results of this study may provide useful information concerning the high incidence of secondary bacterial and yeast infections associated with TPN therapy.

The Growth and Decomposition of *Chloroflexus-Synechococcus* Microbial Mats in Alkaline Thermal Springs. T. PARKIN, J. C. COOK, and W. N. DOEMEL, Wabash College, Crawfordsville, Indiana 47933.—The growth and decomposition of a *Chloroflexus-Synechococcus* microbial mat in a thermal spring located in the Lower Geysir Basin, Yellowstone National Park, Wyoming, was studied with silicon carbide (carborundum) as a neutral substratum. Silicon carbide was placed onto the microbial mat with a template. The increase of mat thickness and Lowry protein above the silicon carbide (growth) was monitored regularly by collecting a coring of the mat over the silicon carbide with a standard cork borer. A second layer of silicon carbide was placed over this new mat when about 2 mm had accumulated. The decrease of mat thickness and Lowry protein (decomposition) between these two layers was then monitored in the same way. From these data, rates of growth and decomposition were determined. In this microbial mat and in other mats in similar pools, the rate of decomposition approximated growth rate so that there was little net accumulation of mat above a "foundation level." At these temperatures, eukaryotes are absent so that prokaryotic microorganisms are responsible for all of the decomposition. This is contrary to a general opinion that eukaryotes, particularly insects, are necessary for decomposition to be significant.

The Structure of *Chloroflexus-Synechococcus* Microbial Mats in Alkaline Thermal Springs. WILLIAM N. DOEMEL, Wabash College, Crawfordsville, Indiana 47933.—The filamentous photosynthetic bacterium *Chloroflexus* (bacteriochlorophylls a and c) independently and in association with the blue-green alga (Cyanobacterium) *Synechococcus* (phycocyanin and chlorophyll a) forms laminated mats in alkaline thermal pools between 55 and 65 C. At night or in the absence of algal photosynthesis, *Chloroflexus* migrates upwards covering the mat with a thin orange-pink layer. *Chloroflexus* also forms a number of differentiated structures on the mat—"nodes," 0.5-1.0 mm conical projections; colonies—circular, flat orange growths with green centers; and streamers. Ultra-thin sections of microbial mat show vertical, horizontal, and alternating vertical and horizontal arrays of *Chloroflexus*. The laminated structure of the mat is similar to fossil stromatolites and suggests that photosynthetic bacteria, like *Chloroflexus*, may have formed some of these structures rather than filamentous blue-green algae.

Fluorescent Whitening Agents: Effects on Selected Algae. A. BROOKS and W. DOEMEL, Wabash College, Crawfordsville, Indiana 47933.—The impact of fluorescent whitening agents (optical brighteners) on the growth and photosynthesis of *Chlorella* and *Microcystis* was investigated.

Polar optical brighteners bound strongly to cellulose and to algae with cellulose walls while semi-polar and non-polar optical brighteners did not bind to most algae. Since most optical brighteners absorb in the ultra-violet and fluoresce between 400-5—nm, algal photosynthesis could be enhanced; however the uptake of ^{14}C -bicarbonate by *Chlorella*, *Microcystis*, and natural populations of algae was unaffected by either polar or non-polar optical brighteners. Since optical brighteners bind to the wall of *Chlorella*, growth might be inhibited by disruption of transport mechanisms; however, the growth of *Chlorella* was not inhibited by polar optical brighteners at 2.5 ppm. Since natural concentrations of optical brightener range between 0.0015 and 0.015 ppm, these observations suggest that natural concentrations of optical brighteners would have no effect upon natural algal populations.

Effects of Defined Microflora on Bile Acids of Gnotobiotic Rats. D. MADSEN, M. WAGNER, and B. WOSTMANN, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556.—The bile acids (BAs) hyodeoxycholate (HDC) and ω -muricholate (ω -MC) are both absent from germ-free (GF) Lobund/Wistar rats, but in the conventional animal they comprise about 50% total excreted (fecal) BAs. Body pools of both neutral and acid sterols are larger in GF than in CV rats. Since BA excretion is the major pathway for removal of body cholesterol, and because HDC and ω -MC appear *not* to be greatly reabsorbed during passage through the gut, we have postulated that in the CV rat the potential to form these two BAs serves to control body cholesterol pools.

We have become interested in identifying the bacterial species responsible for initiating production of HDC and ω -MC, presumably from the primary BA, β -MC. More specifically, we wanted to know if initiation of this pathway is a property of many or of only a selected few of the bacterial species in the rat gut.

One experiment involved GF rats which were accidentally contaminated with a single species of a diphtheroid. This organism partially deconjugated all BAs present, and formed keto BAs. However, neither HDC, ω -MC, or deoxycholate were found in feces.

Another experiment made use of rats associated with a defined hexaflora developed at the Lobund Laboratory. The hexaflora contained: *Lactobacillus brevis*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Bacteriodes fragilis* var. *vulgatus*, and *Torulopsis* sp. Although fecal BAs were partially deconjugated, again no deoxycholate, HDC, or ω -MC were found, nor were derivatives of cholesterol; keto-acids in feces were within the range found in CV rats.

These results indicate that the capacity to produce the secondary BAs mentioned above is found in relatively few bacterial species of the rat gut, especially when compared to the capacity to deconjugate and to form keto BAs. (Supported by the National Institutes of Health and the Fannie E. Rippel Foundation.)

Inactivation of Microorganisms by Simultaneous Treatment with Ozone and Ultrasonics. GARY BURLESON and MORRIS POLLARD, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.—The

effects of ozone, ozone and sonication, or sonication on microorganisms with public health significance were investigated. Microorganisms suspended in phosphate-buffered saline were rapidly inactivated by treatment with ozone. Microorganisms suspended in secondary effluent from a wastewater treatment plant required longer contact times with ozone for complete inactivation. Simultaneous treatment by ozonation and sonication reduced the contact time for complete inactivation of microorganisms in secondary effluent. Since sonication alone or sonication with oxygenation did not inactivate test bacteria, the enhanced inactivation by simultaneous treatment with ozone and ultrasonics is interpreted as a synergistic effect.

Decontamination of Gnotobiotic Mice Monoassociated with *Candida albicans*. MORRIS WAGNER and KUNWAR K. SRIVASTAVA, Lobund Laboratory, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556.—Candidiasis is a common health hazard in patients undergoing physical and/or drug treatment associated with organ transplantation and neoplastic diseases. It is also a problem in infectious disease patients receiving broad spectrum antibacterial antibiotics that alter the normal intestinal flora. In the present investigation, germfree AKR mice were monoassociated with *Candida albicans* and used as a model to determine whether such mice could be decontaminated successfully and returned to their earlier germfree state by oral treatment with amphotericin B. This model could provide useful information for the control of candidiasis in patients.

C. albicans became well established in gnotobiotic mice (\log_{10} of number per gram of organ plus contents averaged 7.9 and 7.7 in stomach and cecum respectively). Direct fecal smears as well as impression smears of the stomach and cecal mucosal surfaces revealed yeast-phase cells, many with germ tubes, but no mycelium. No illness or mortality was observed over a four-week acclimatization period. The mice were then given *ad libitum* amphotericin B dissolved in the drinking water. At levels of 0.1 and 0.2 mg/ml, the number of *C. albicans* decreased but the organism was not eliminated completely. However, 0.3 mg/ml was sufficient to decontaminate the mice completely and return them to the germfree state. Residual amphotericin B was detected in the feces of mice receiving the high 0.3 mg/ml level only. These mice tested germ-free after 4 weeks of treatment at this level and continued to test germ-free until the termination of the experiment ten weeks after the antibiotic had been discontinued and replaced by plain drinking water.

The Use of Liposomes to Enhance the Antiviral Activity of Polyuridylic Acid. THOMAS J. TINGHITELLA and CHARLES F. KULPA, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556.—Recent studies with artificial multilamellar phospholipid vesicles (liposomes) which are similar to natural membranes have shown their potential as general carriers of therapeutic agents. The properties of liposomes may in effect enhance the activity of the enclosed chemotherapeutic agent.

Liposomes themselves are easily prepared. Phospholipids are dried in a round-bottom flask using a rotary evaporator; the material to be

enclosed is dissolved in water, then added to the dried lipids. Vigorous agitation results in the spontaneous formation of these lipid vesicles. Cholesterol may be added to the phospholipids to increase the stability of the structures produced. The presence of long chain cations or anions increases the spacing between vesicle bilayers and adds a net negative or positive charge.

Using either neutral or charged liposomes we attempted to enhance the inhibitory activity of the synthetic RNA polymer, polyuridylyate against vesicular stomatitis virus. *In vitro* poly U is an effective inhibitor of the polymerases needed for replication of RNA viruses. However, little or no activity of free poly U is demonstrable against the replication of vesicular stomatitis and other RNA viruses in cell culture. This may be due to rapid extracellular degradation or the inability of the cell to take up the polymer.

Our studies have shown that poly U can be protected from degradation when it is enclosed in liposomes. Negatively charged liposomes enclosing poly U were found to be ineffective against VSV replication while liposomes with a net positive charge and containing poly U had an inhibitory effect on VSV replication.

Functional Bile Acid Patterns in Gnotobiotic Mammals. BERNARD S. WOSTMANN and MARGARET H. BEAVER, University of Notre Dame, Lobund Laboratory, Notre Dame, Indiana 46556.—Many gastro-intestinal studies would benefit if the experimental model included the possibility of control and definition of the microbial variable. These include studies on digestion and absorption, on intestinal motility and excretion, and aspects of experimental surgery.

Many functional aspects of the gut may be affected by the qualitative and quantitative composition of intestinal bile acids (BA). These include solubilization of lipids, lipase action, energy dependent transport, renewal rate of the intestinal mucosa and its cell age-dependent enzyme systems, etc. We have compared germfree (GF) versus conventional (CV) states of mammals (rat, mouse, gerbil, pig, dog, rabbit, and human) to determine the effect of microflora on BAs.

BA patterns of CV rats and mice differ greatly from GF, due to the production of the primary BAs cholic and β -muricholic acids, and to the presence of the large rodent cecum. The gerbil and rabbit produce only chenodeoxycholic and cholic acids, but possess sizeable ceca, and show major differences in GF and CV functional BA patterns. This is also true of the pig which produces cholic and hyocholic acids, although the microbial reservoir (cecum) is virtually absent. The dog, with cholic acid as the major primary product, shows the smallest qualitative and quantitative difference in functional BA patterns between the GF and CV states.

These observations suggest the gnotobiotic dog as a model of choice for studies of gastro-intestinal function which require control of the intestinal microflora. While a quantitative resemblance exists between BAs of dog and human, the qualitative human BA pattern resembles that of the gerbil more than of the dog. (NIH and Fannie E. Rippel Foundation.)