

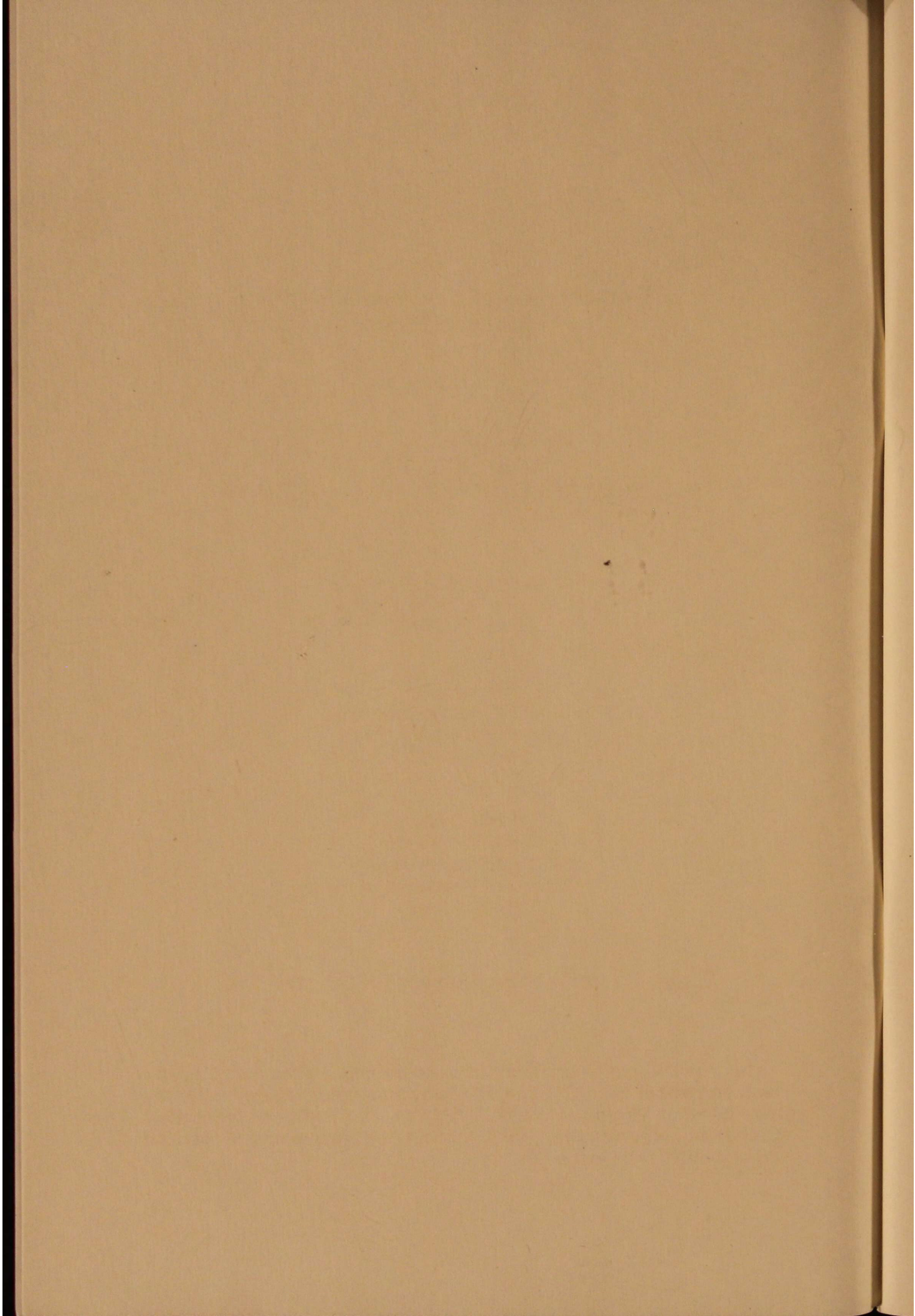
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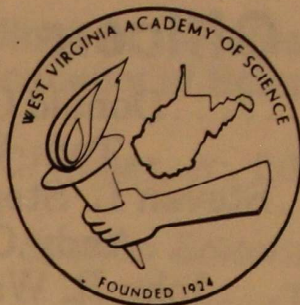
Proceedings of the West Virginia Academy of Science 1992



**Abstracts of papers of
the Sixty-Seventh Annual Session**







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**Abstracts
of the
1992 Annual
Meeting**

Biology

CARL F. WELLSTEAD, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136.

A survey of Permo-Carboniferous tetrapods in West Virginia.

Impressively complete and well preserved specimens of Paleozoic tetrapods are known from Upper Mississippian rocks of West Virginia. These represent some of the oldest land vertebrates known from North America. Fossil tetrapods are also known from dozens of localities in Upper Pennsylvanian and Lower Permian strata of the state. These younger specimens are frequently not nearly so complete as the noted Mississippian examples, but do document a much wider variety of terrestrial vertebrate species. Puzzling is the current lack of any record of tetrapods from Allegheny and older Pennsylvanian sediments. Why are there no reports of such remains from this portion of West Virginia's coal measures? Are the fossils absent or have too few been searching for them?

JEFFREY A. ANDERSON, DEBORAH A. LEONARD, Dept. of Biology, West Virginia University, Morgantown, West Virginia 26506 and LEAH L. FRYE, KEVIN P. CUSACK, Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180. Oxylanosterol Inhibitors of HMG-CoA Reductase.

Coronary Heart Disease (CHD) is currently the nation's leading killer. The underlying cause of CHD is atherosclerosis, and elevated blood serum cholesterol has been established as a major risk factor in the development of atherosclerosis. Reduction of serum cholesterol levels may be achieved by diet modification and/or drugs which inhibit cholesterol production. Inhibition of the rate-determining enzyme, 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR) reduces cholesterol biosynthesis. Oxylanosterols, natural precursors to cholesterol, are effective inhibitors of HMGR and at least some of these compounds are thought to act by post-transcriptional mechanisms. This is in contrast to oxysterols which are intermediates in the production of bile salts and steroid hormones and act primarily at the level of gene transcription. We have screened a variety of 15-substituted oxylanosterols for inhibition of HMGR activity and characterized their mechanism of action in Chinese hamster ovary (CHO) cells and a lanosterol demethylase deficient mutant cell line. The proton at C-15 is required for the removal of the C-32 methyl group of lanosterol. The presence of a functional group at C-15 should prevent the metabolism of these compounds. Enzyme assays and quantitative immunoblot analysis have been used to identify compounds which affect HMGR by altering gene expression as indicated by parallel decreases in enzyme activity and immunoreactive protein. Five oxylanosterols with various substitutions at the 15 position have been screened, and one compound has been extensively characterized. F11, an oxylanosterol with an oxime at C-15, is a potent inhibitor of HMGR activity with parallel decreases in enzyme activity and immunoreactive protein. Northern blot

analysis and measurements of HMGR synthesis and degradation rates indicate that this compound is a post-transcriptional regulator acting primarily at the level of translation.

KIMBERLY S. CROSS, DEBORAH A. LEONARD, Dept. of Biology, West Virginia University, Morgantown, West Virginia 26506 and LEAH A. FRYE, KEVIN P. CUSAK, Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180. Dual Action Inhibitors of Cholesterol Biosynthesis.

Atherosclerosis, a state leading to coronary heart disease, is attributed to the accumulation of cholesterol-containing LDL particles in the arterial wall. Understanding the regulation of cholesterol biosynthesis is important in the development of hypocholesterolemic drugs. The rate-limiting enzyme of *de novo* cholesterol biosynthesis is HMG-CoA reductase (HMGR), an enzyme which is regulated both transcriptionally and post-transcriptionally. Oxysterols, which are formed as intermediates in cholesterol biosynthesis, may be natural regulators of cholesterol biosynthesis. In this project, 7-substituted oxysterols were screened for inhibition of HMG-CoA reductase activity in mammalian cells. The mechanisms of action of several compounds, which were identified as potent inhibitors of HMGR, were characterized. Quantitative immunoblot analysis was used to determine the amount of HMGR immunoreactive protein. All of the compounds tested inhibited HMGR activity by regulating gene expression. Incorporation of [¹⁴C]acetate into non-saponifiable lipids and enzyme assays showed that these compounds also act as direct inhibitors of lanosterol demethylation. Immunoprecipitation of radiolabelled proteins was used to measure the effects of oxysterols on HMGR synthesis and degradation. HMGR mRNA analysis was used to determine if any of the compounds act by post-transcriptional mechanisms. Post-transcriptional regulatory compounds decrease enzyme activity and immunoreactive protein without lowering HMGR mRNA levels.

MARLISE FRANKE and JOSEPH B. MORTON, Dept. of Plant Pathology and Environmental Microbiology, West Virginia University, Morgantown, West Virginia 26506. Ontogenesis of two arbuscular mycorrhizal fungi and its phylogenetic implications.

Phylogenetic relationships have been hypothesized for the symbiotic endomycorrhizal fungi, which then were incorporated into a new classification (Glomales, Zygomycetes). Stable morphological characters were used in cladistic analysis. However, accuracy of the cladistic method depends on recognition of characters having similar origin (homologies) and of polarity (primitive X derived). The ontogenetic method is one approach to obtain data on homology and polarity. The ontogenesis of two Glomalean fungi, Scutellospora dipapillosa and S. pellucida, was followed in two host plants, sudangrass and red clover. The development of colonization and the differentiation of spores were followed in time (5-13 weeks after seeding of hosts in 150 cm³ "cone-tainers"). Arbuscules and auxiliary cells were formed early (5 weeks) in all treatments. New spores appeared at 6 weeks. Both colonization and spore production reached their peaks at different times, depending on fungus-host combinations. Subcellular differentiation in spores of S. pellucida was separated into five discontinuous stages. Six stages

were found in spores of *S. dipapillosa*. Stages of differentiation in spores of both fungi were constant on both host species. Stages 1 and 2 were homologous in *S. pellucida* and *S. dipapillosa*, after which different subcellular components developed in each fungus. In *S. dipapillosa* a unique stage early in the developmental sequence was discovered. It appears to be a non-disruptive addition, because it did not alter subsequent stages of development. These ontogenetic patterns show that these two species are not as closely related as previously hypothesized. Based on this work, many of the same mechanisms of development found in more complex eukaryotic systems occur with single cells of these fungi.

LEONORA J. GROVES, RANDI B. WEISS, and
PATRICIA E. GALLAGHER, West Virginia
University, Department of Biology,
Morgantown, WV 26506. Detection of
Adenine Photoproducts in Human
Lymphoblast DNA Using a Bacterial
Endonuclease as a Probe.

While the ultraviolet mutagenesis and carcinogenesis of pyrimidine photoproducts have been extensively investigated, DNA purines were long assumed to be relatively inert to photochemical modification. However, several purine photoproducts have been detected *in vitro* in ultraviolet irradiated dinucleotides, polynucleotides and DNA, including two distinct adenine dimers. Here we describe the purification of a bacterial endonuclease which incises ultraviolet irradiated DNA at sites of adenines. This adenine-photoproduct incising activity was then used as a probe to detect the formation of enzyme-sensitive sites in DNA extracted from ultraviolet irradiated human cells.

Using ultraviolet irradiated poly(dA)-poly(dT) labeled with [^3H]-dATP as a substrate, an enzymatic activity which recognizes adenine-containing photoproducts was isolated from *E. coli*. This enzyme was separated from other known bacterial endonucleases which incise ultraviolet irradiated DNA by ion-exchange chromatography. The partially purified enzyme specifically released radiolabeled material from the purine strand, showing no activity against ultraviolet irradiated polymer radiolabeled in the pyrimidine strand. In addition, the enzyme did not release TCA-soluble material from ultraviolet irradiated poly(dA-dG)-poly(dT-dC) radiolabeled with [^3H]-dATP. This suggests that the enzymatic activity is directed against adenine-containing photoproducts in ultraviolet irradiated polynucleotide and not toward structural distortions of the substrate induced by the formation of bi-pyrimidine photoadducts. Analysis of data generated from a DNA sequencing assay showed that the partially purified enzyme incised ultraviolet irradiated DNA at sites of adenine

residues in regions of the substrate containing multiple adjacent adenines. These data suggest that the enzymatic activity is directed against adenine-containing photodimers in DNA.

The adenine-photoproduct incising activity was used as a probe to detect the formation of purine photoproducts *in vivo* in human lymphoblasts. Human lymphoblasts were treated with increasing, non-lethal doses of ultraviolet irradiation and damaged DNA was isolated. Following incubation of the DNA with the partially purified enzyme, the formation of enzyme-sensitive sites in total genomic DNA was determined by alkaline sucrose density gradient centrifugation. Using this method, the rate of *in vivo* repair of these adenine photoproducts is currently under investigation. (Supported by NIH grant CA-47457 and WVU Senate Grant).

JOHN E. HALL, Department of Microbiology and Immunology, and JAMES W. AMRINE, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506. Distribution of *Ixodes* ticks biting humans in West Virginia: Implications for Lyme disease.

In attempts to identify ticks attacking humans in West Virginia, four species of *Ixodes* have now been recorded. *Ixodes cookei* Packard, commonly a parasite of woodchuck and fox squirrel, has been removed from humans on 65 occasions. These reports are from 26 counties, well distributed throughout the state. Most of these ticks were attached and feeding; nymphs were encountered most frequently (60 reports), whereas adults were found on four occasions and larvae on one occasion. *Ixodes dentatus* Marx, usually found on rabbits, has been recovered from humans on eight occasions, and from six different counties. Seven of these specimens were nymphs, the remaining specimen was a larva. A single larva of *Ixodes texanus* Banks was found attached to a child living in Upshur County, and an adult female of *Ixodes dammini* Spielman, Clifford, Piesman, and Corwin was removed from the scalp of an individual in Jefferson County. One of the specimens of *Ixodes cookei*, an adult female removed from a woman living in Boone County was opened and the gut was examined using a fluorescent antibody test for *Borrelia burgdorferi*, the agent of Lyme disease; it was found positive. Nine other specimens of

I. cookei which were examined by dark field microscopy or by fluorescent antibody testing were apparently negative. Two of the *Ixodes cookei* nymphs were associated with target lesions resembling those typical of Lyme borreliosis.

Though 44 cases of Lyme disease were reported from throughout West Virginia in 1991, the usual vector, *Ixodes dammini*, has been collected only from Jefferson County in the Eastern Panhandle. Since most of the *Ixodes* ticks biting humans in West Virginia are *I. cookei*, and since this tick has been found to harbor Lyme disease organisms and has been associated with target lesions, we suggest that this species may be serving as a Lyme disease vector and that woodchuck and fox squirrels rather than deer and white footed mice may be serving as reservoirs of the ticks and spirochetes in some areas. *Ixodes dentatus* suggests a cottontail rabbit reservoir.

TED HIGSON AND MICHAEL KOTARSKI

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Molecular Analysis of Star and asteroid of
Drosophila melanogaster

The asteroid gene plays a central role in the development of *Drosophila melanogaster*. Its proper expression is essential for the development of many of the insect structures, most notably the compound eye. Recessive mutations of the asteroid gene disrupt the proper development of the hexagonal close-packed array of more than 800 ommatidia that compose the eye. Star mutations are dominant and when present in only a single copy in the genome produce eye defects similar to asteroid mutations. Homozygous Star individuals die in early embryogenesis due to developmental defects. Many independent Star mutations have been produced over the past forty-five years and each of these mutations is associated with a chromosome aberration. Star mutations interact with asteroid mutations in a complex manner and were thought to be defects within a gene with a similar, but distinct developmental function. DNA from the region of the Star-asteroid locus has been cloned by P element transposon tagging. Southern blot analysis of several Star and asteroid mutations indicate that Star mutations are alleles of asteroid. The data suggest that dominant Star mutations are the result of the complete absence of asteroid gene function, while recessive asteroid mutations are the result of a decrease in gene function.

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CONTAMINANT LEVELS IN RACCOONS (PROCYON LOTOR)
AT WHITEOAK CREEK FLOODPLAIN, OAK RIDGE, TENNESSEE.

Raccoons were trapped from the Whiteoak Creek Floodplain (WCF) which is part of the Oak Ridge Reservation (ORR) at the Oak Ridge National Laboratory, Oak Ridge, Tennessee. This area has been used as a burial ground for contaminated and radioactive wastes since the 1940's. Mercury, PCBs, ¹³⁷Cs and ⁹⁰Sr were the contaminants of primary concern. Samples of hair, liver and bone from the ORR were analyzed for these contaminants. Samples of hair from raccoons trapped from WCF were analyzed for ¹³⁷Cs and mercury. Samples from an off-site, uncontaminated location are to be analyzed for comparison.

ULRIKE KLOTZ, RANDI B. WEISS, and
PATRICIA E. GALLAGHER. West Virginia
University, Department of Biology,
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Release of Methylated Pyrimidines by a
Human DNA Glycosylase.

The DNA of all cells is continuously subjected to damage caused by environmental agents as well as intermediary metabolites. Methylation of cellular DNA has been shown to produce a number of base modifications. The most predominant lesions formed are the N-methylpurines, 7-methylguanine and 3-methyladenine, while the methylpyrimidines are produced in lesser quantities.

Studies focusing on the repair of methylpyrimidines have shown that enzymatic activities in *Escherichia coli* recognize and remove these lesions from methylated substrates. Excision of methylpyrimidines is initiated by the DNA glycosylase which also releases 3-methyladenine and 7-methylguanine lesions from DNA. However, it has been shown that the specificity of the bacterial repair enzymes isolated from *Escherichia coli* differ greatly from the repair enzymes isolated from mammalian cells. No enzyme has yet been isolated from eukaryotic sources that can measurably repair or remove methylpyrimidines. Since it has been shown that several types of methylpyrimidines in DNA will mispair upon DNA replication, these adducts, if not excised from damaged cellular DNA, may contribute to mutagenesis and carcinogenesis in mammalian

cells. Therefore, we have investigated the mechanism by which mammalian repair enzymes remove methylpyrimidines from damaged DNA.

In order to identify and characterize a human DNA repair activity which recognizes methylated cytosines, a base release assay was used. Poly(dGdC)-poly(dGdC), a synthetic polymer radiolabeled with [³H]-dCTP by nick translation, was subjected to methylation by dimethyl sulfate. 3-methyladenine-DNA glycosylase, partially purified from human lymphoblasts, was incubated with methylated and unmethylated polymer. Release of TCA-soluble reaction products was linear with respect to protein concentration and time of incubation. There was no release of TCA-soluble material from unmethylated polymer. Heat inactivation experiments confirmed the enzymatic nature of the reaction.

These results suggest that mammalian cells contain an enzyme that releases methylcytosines from methylated poly(dGdC)-poly(dGdC). The identification of the specific methylpyrimidines released by the human enzyme is currently under study. (Supported by NIH grant CA-47457)

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and G. K. BISSONNETTE, Dept. of Plant
Pathology and Environmental Microbiology,
West Virginia University, Morgantown, West
Virginia 26506. Microbiology of point-of-
use filters and filter-treated rural
groundwater supplies.

Water quality was monitored for 24 rural, domestic, groundwater supplies treated with point-of-use (POU) powdered activated carbon (PAC) filters. This field study was conducted to determine how home treatment might impact the microbiological quality of private drinking water supplies. Total coliform and heterotrophic plate count (HPC) analyses were performed on untreated, PAC-treated, and overnight stagnant PAC-treated water samples. Results indicate that the HPC were generally elevated by 0.86 and 0.20 orders of magnitude, respectively, for spring and well water systems in PAC-treated effluents following an overnight stagnation period as compared with HPC in untreated influents. The number of heterotrophs in PAC-treated effluents could be significantly ($p < 0.0145$ for springs; $p < 0.0001$ for wells) reduced below influent levels, however, after flushing the POU device. PAC treatment significantly ($p < 0.0001$) reduced the number of coliforms in product waters; however, coliforms were still detected in filter effluents. Seasonal variations were evident in microbiological counts from spring, but not well, water systems. It appears that PAC treatment, other than following stagnant periods of water usage, does not compromise the microbiological quality of private drinking water supplies.

S. CRAIG STAMM, BAO-ZHEN ZHONG, TONG-MAN ONG, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505 and E.C. KELLER JR., Dept. of Biology, West Virginia University, Morgantown, West Virginia 26506. Examination of the mutagenicity and possible aromatic amine/nitroarene content of two coal dust extracts in the Ames Salmonella microsomal assay system.

The mutagenic activity of both non-nitrosated and nitrosated coal dust extracts of a West Virginia (bituminous) and a New Mexico (subbituminous) coal were studied using the Ames Salmonella microsomal assay system. Two tester strains were utilized: TA98, a conventional Ames test strain which detects frameshift mutagens and YG1024, a TA98 derivative specifically designed for enhanced sensitivity to aromatic amines/nitroarenes. No significant mutagenic activity was detected in the non-nitrosated extracts by either strain with or without metabolic activation. Significant direct-acting mutagenic activity was found in the nitrosated extracts from both types of coal on each tester strain. A comparison of the mutagenic responses between strains suggests that the nitrosated New Mexico coal dust extract may also contain an indirect-acting chemical mutagen of the aromatic amine/nitroarene class. Since the two coals examined were from different ranks and locations, it is difficult to define the factors leading to the additional presence of this class of mutagen in the New Mexico coal extract and its absence from the West Virginia coal extract. However, further comparison studies may be of value in assessing the potential risks to miners of exposure to coal dusts and may be relevant to the increased incidence of gastro-intestinal cancer existing in this industry.

LAUREL A. STOLLAR, Department of Medical Technology, West Liberty State College, West Liberty, WV 26074, A Comparison of Isolation techniques of Listeria monocytogenes.

The highly selective, newly developed Al-Zoreky-Sandine Listeria Medium was compared to the standard FDA procedure for the isolation of Listeria monocytogenes. Environmental samples were run repeatedly over a three month period, and the results show the ASLM agar to be effective in isolating L. monocytogenes from mixed samples.

V. ULRICH and P. MULLEN, Div. of Plant and Soil Sciences, W.V.U., Morgantown, W.V. 26506-6108. The influence of three antibiotics and two temperatures on *Medicago sativa* callus development, induction and regeneration.

The root and hypocotyl derived cultured cells and calli of alfalfa, *Medicago sativa* (cv. 'Regen S') were employed to investigate the influence of carbenicillin chloramphenicol and tetracycline on calli growth and embryogenesis under standard and stressed conditions. Carbenicillin removed bacterial contamination, promoted calli growth and increased embryogenesis on maintenance medium. Carbenicillin also had the highest percentage of embryonic loci after 30 days of growth. Chloramphenicol produced a reduction or cessation of growth under standard and stressed conditions. Chloramphenicol treated cultures also had lower numbers of embryonic loci at thirty days than did either the control or carbenicillin treated cultures. Tetracycline was lethal to all cells and calli under all experimental conditions.

JAGAN V. VALLURI, H. W. ELMORE, JULIE C. HANNAH and DOUGLAS B. CHAMBERS, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Protein synthesis in Sandalwood callus cultures exposed to drought and salt stress.

Effects of mannitol - induced water stress and NaCl-induced salt stress on protein synthesis were investigated in heterotrophic callus cultures of sandalwood (*Santalum album* L). A set of cultures were transferred to a medium containing 0-15% mannitol to provide water potentials between -0.4 and -2.5 MPa. A second set of cultures were exposed to media containing different levels of NaCl (0 - 3%). Proteins were sampled after 2, 4, 8, 16, 24 or 48 h of exposure to stress. Cells were able to tolerate and grow in mannitol up to 9.1% (0.5M) or NaCl up to 1% (0.17M), but the relative overall growth was about 1/6 of the control. Proteins were visualized after separation by one-dimensional SDS-polyacrylamide gel electrophoresis and staining by silver. No detectable differences were apparent in the abundant, steady state protein populations during initial time periods of mannitol and salt-induced stress. Random losses of individual proteins occur after 48 h at 2.5 MPa and 3% NaCl. Most notable difference was the enhancement of low molecular weight proteins (15 - 30 kD) in calli exposed for 24 and 48 h at 10, 12 and 15% mannitol. Calli exposed to 0.05M, 0.10M and 0.15M salt concentrations showed specific enhancement of a single low molecular weight protein (30 kD) after 16 and 24 h incubation. Water and salt stress seem to signal the induction of specific proteins. If these proteins have a role in stress tolerance at the cellular-level, then identification of the specific genes would open up the way to the genetic engineering of drought/salt resistance in trees.

RANDI B. WEISS and
PATRICIA E. GALLAGHER.
West Virginia University, Department of
Biology, Morgantown, WV 26506. *In vivo*
Repair of Oxidative Damage in the DNA of
Human Lymphoblasts.

Oxidation of the DNA bases is caused by a variety of physical and chemical agents in the environment as well as intermediates of normal cellular metabolism. Mechanisms for the repair of many of these lesions have been well defined in both bacterial and mammalian systems. Repair of oxidized pyrimidines from damaged DNA is initiated in mammalian cells by the action of the redoxendonuclease, the counterpart to *E. coli* endonuclease III. Both the bacterial and mammalian enzymes have DNA glycosylase activities, releasing ring-saturated or ring-fragmented pyrimidines from DNA damaged by chemical oxidation as well as ionizing and ultraviolet irradiation, leaving an apyrimidinic site. An associated apyrimidinic endonuclease activity incises the DNA at the resultant base loss site.

We have investigated the formation and *in vivo* repair of oxidized pyrimidines in human lymphoblasts following treatment of cultured cells with increasing concentrations of hydrogen peroxide. The extent of DNA damage in total genomic DNA was determined by alkaline sucrose density gradient centrifugation using *E. coli* endonuclease III as a probe. A concentration of 5 mM hydrogen peroxide resulted in detectable levels of enzyme-sensitive sites with greater than 50% cell survival up to 24 hours after removal of the damaging agent. This method was also used to determine the *in vivo* rate of repair of ring-saturated pyrimidines in oxidized DNA. Human lymphoblasts were treated with 5 mM hydrogen peroxide for 30 minutes at 37°C. After removal of the damaging agent, the DNA was isolated from aliquots of cells harvested at various time intervals and incubated with saturating concentrations of *E. coli* endonuclease III. Analysis of alkaline sucrose gradients showed that excision of oxidized pyrimidines from total genomic DNA occurs rapidly *in vivo* and is essentially complete within 3 hours.

To examine the *in vivo* repair of ring-saturated pyrimidines in defined sequences of human DNA, an assay based on the polymerase chain reaction was used. By this method, enzyme-generated strand breaks result in the formation of truncated products as demonstrated by the loss of full-length amplified product. Following treatment of human lymphoblasts with a non-lethal concentration of oxidizing agent, the cells were incubated in fresh medium and aliquots were harvested at various times. Segments of the human genes for dihydrofolate reductase, creatine kinase, and elongation factor 1 were amplified using limiting concentrations of the DNAs extracted. The radiolabeled, amplified products of each polymerase chain reaction were quantified. Repair of the oxidized pyrimidines in all the defined regions of the genome studied was essentially complete within three hours. These results are in contrast to the rate of removal of bulky adducts from nontranscribed regions of cellular DNA which appears to be a slower

process. It is possible that condensed regions of the genome are more accessible to the redoxendonuclease, a small, single-chain polypeptide, than to a multi-protein complex which is presumably required to repair bulky adducts in cellular DNA. (Supported by NIH grant CA-47457 and WVU Senate Grant).

PAUL W. WRIGHT, RANDI B. WEISS, and
PATRICIA E. GALLAGHER. West Virginia
University, Department of Biology,
Morgantown, WV 26506. *In Vivo* Repair of
N-Methylpurines in Human Nuclear and
Mitochondrial DNA.

Although most cellular DNA is present in the nucleus, mitochondria contain their own DNA that codes for ribosomal and tRNAs as well as thirteen of the genes that code for the four enzyme complexes of oxidative phosphorylation. In recent years, studies have shown that major deletions and point mutations of mitochondrial DNA cause a number of human neuromuscular diseases, including mitochondrial myopathies and encephalopathies, and Leber's hereditary optic neuropathy. Since mitochondrial DNA is not complexed with histones or histone-like proteins, it has been suggested that the mitochondrial DNA may be more susceptible to damage by chemical and physical agents. Studies from a number of laboratories suggest that excision repair of damaged base sites in mitochondrial DNA may occur, however, limited information exists on removal of damage from mitochondrial DNA. We have employed an assay based on the polymerase chain reaction to examine the *in vivo* formation and repair of methylpurines in defined segments of mitochondrial and nuclear DNA.

Suspension cultures of human lymphoblasts were incubated with nonlethal concentrations of dimethyl sulfate for 30 minutes at 37°C. The predominant DNA base lesion formed by this treatment is 7-methylguanine which accounts for more than 70% of the total DNA damages. Following treatment with dimethyl sulfate, the cells were resuspended in fresh culture media and incubated at 37°C to allow repair of the damaged DNA. At various times, the cellular DNA was extracted and analyzed for the presence of methylpurines in defined segments of nuclear and mitochondrial DNA.

A polymerase chain reaction assay was developed to detect the *in vivo* formation and rate of repair of methylpurine lesions in nuclear and mitochondrial DNA. Defined segments of human cellular DNA, isolated after dimethyl sulfate treatment of cultured lymphoblasts, were amplified using the polymerase chain reaction. Apurinic sites were generated due to unstable methylpurines which caused termination of *in vitro* replication resulting in the formation of truncated products. Thus, as the methylpurines in the

cellular DNA are repaired *in vivo* the amount of amplified product increases. Segments of the human nuclear genes for dihydrofolate reductase and elongation factor I and two mitochondrial DNA segments that included the coding region for lysyl t-RNA and a subunit of nicotinamide adenine dinucleotide dehydrogenase, respectively, were amplified using limiting concentrations of DNA. Radiolabeled, amplified products of each polymerase chain reaction were quantified. Data analysis showed that the formation of methylpurines was greater in the mitochondrial sequences studied as compared to the nuclear gene segments amplified. Additionally, it was found that methylpurines are removed from mitochondrial DNA sequences at a rate comparable to that of the nuclear gene fragments. These data suggest that nuclear encoded DNA excision repair enzymes can traverse the mitochondrial membrane and actively repair mitochondrial DNA damage. (Supported by NIH grant CA-47457)

Botany

DAN CHAFFIN, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755 and ROBERT DEAL, Dept. of Science, Shawnee State University, Portsmouth, Ohio 45662. Apical Anatomy of Cultivated Dahlia, *Dahlia Variabilis* Desf., cult. Amber Queen.

A plot of Cultivated Dahlia supported plants with decussate phyllotaxy, typical for Dahlia and alternate leaf arrangement, atypical for the genus. Apical meristems of axillary shoots were excised, fixed in FPA, embedded in plastic and paraffin, and sectioned and stained with toluidine blue and Safranin-Fast Green. Longitudinal and transverse sections revealed a normal structure and leaf development in lateral meristems from all plants, and the atypical alternate leaf arrangement is attributed to mechanical injury to the primary stem apex. Photomicrographs show developing stages of uniseriate trichomes, anomocytic stomata, axillary buds, schizogenous secretory canals, vasculature, floral meristems, and overall structure of the vegetative meristem.

TARA DUBEY, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136, STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, and PAMELA J. EDWARDS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287. The effect of stream pH on the distribution patterns of aquatic fungi.

Aquatic Phycomycetes (chytrids and water molds) are common inhabitants of freshwater streams, where they occur as saprophytes or parasites on a wide variety of substrates, including algae, other aquatic fungi, aquatic plants, plant detritus, and microscopic aquatic animals. Aquatic Hyphomycetes (imperfect fungi) play an important role in increasing the palatability and nutritional quality of plant detritus for invertebrate grazers, while at the same time enzymatically degrading the detritus. The role of aquatic Hyphomycetes is especially important because many aquatic invertebrate grazers incapable of digesting cellulose and lignin are able to consume fungal biomass generated by detritus-decomposing fungi. Fungal activity appears to be most important during the initial stages of decomposition. The distribution patterns of the aquatic fungi occurring in five streams located on or near the Fernow Experimental Forest in Tucker County, West Virginia, were studied during the 1991 field season. Water pH averaged >5.5 in two of the streams, whereas the others were relatively more acidic (average pH = 4.2, 3.9 and 3.2, respectively). Sampling methods used included membrane filtration of stream water, litter bags, and "baiting" with various types of organic material. For aquatic Hyphomycetes, both the total number of taxa and the mean number of conidia per 1000 ml of water were lower in the more acidic streams, whereas the number of taxa of Phycomycetes varied little among the five streams. Red oak (*Quercus rubra*) litter was colonized by an average of 14.6 taxa of aquatic Hyphomycetes in the five streams, sugar maple (*Acer saccharum*) litter by 12.6 taxa, and mixed red maple (*A. rubrum*) and beech (*Fagus grandifolia*) litter by 10.6 taxa. For all three types of litter, numbers were generally higher for the less acidic streams and lower for the more acidic streams. (Supported in part by funds provided by the USDA Forest Service.)

WM. HOMER DUPPSTADT, Department of Biology
West Virginia University, PO BOX 6057,
Morgantown, WV 26506-6057. Updates on the
Vascular Flora of West Virginia. VIII.

The new species of plants reviewed and added to the flora
of West Virginia are as follows: Sporobolus neglectus
Nash and Astranthium integrifolium (Michx.) Nutt.

CHARLES KYLE, MARCIA HARRISON, Dept. of
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Huntington, WV 25755
ANALYSIS OF CELL WALL PROTEINS FROM
GRAVITSIMULATED PEA STEMS

Gravitropism is the plant's response to a change in orientation. When plants are turned on their side for a specific time period, the stems become negatively gravitropic (i.e. curve up). This growth response is due to a difference in the growth rate of the lower side compared to upper side of the stem. Cellular growth in plants depends on the expansion of the cell wall due to both synthesis and loosening of the fibers. We have observed enzyme changes which alter cell wall growth are also affected by gravitropism. Therefore, our objective was to evaluate protein/enzyme changes in the plant cell wall fluid (apoplast) altered by gravistimulation. Specifically, we investigated the changes in cell wall proteins and peroxidase activity of dark-grown (etiolated) pea stems before and during gravitropic curvature. Apoplast was collected from etiolated pea (Pisum sativum L. cv. 'Alaska') stem segments at 30 min intervals during gravitropic curvature using a mild centrifugation procedure. According to this procedure, subapical sections of peas were excised under water and washed for 2 min in an aerated water bath. Sections were vacuum infiltrated with cold 50 mM CaCl₂ for 3 min and placed into a holder (modified 20 mL syringe). Apoplast was obtained by centrifugation at 1000 g. This procedure allows for the collection of apoplast with virtually no cytoplasmic contamination. Approximately 3.0 gram samples of segments were taken each time yielding 0.4 to 0.6 mL of apoplast. Peroxidase activity was measured spectrophotometrically by reacting the collected apoplast with substrates, guaiacol and peroxide. Total protein content was analyzed using a dye-binding assay (Bradford method). We found little change in total peroxidase activity in the apoplast due to gravistimulation. However, total apoplastic protein content increased during the later phases of gravitropic curvature. We will also analyze the effect of red light pretreatment on this system. Red light alters cellular growth and affects the kinetics of gravitropic curvature in dark-grown pea stems. SDS-PAGE analysis of apoplastic proteins collected during gravitropic curvature and after red irradiation will be presented.

JOHN C. LANDOLT, Dept. of Biology, Shepherd College, Shepherdstown, West Virginia 25443, STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, and ROSEANN DENSMORE, National Park Service, P.O. Box 9, Denali National Park, Alaska 99755. Dictyostelid cellular slime molds of the Kantishna Hills, Denali National Park and Preserve, Alaska.

The distribution patterns of dictyostelid cellular slime molds (CSM) in soils of fourteen different study sites in the Kantishna Hills of Denali National Park and Preserve were investigated during the 1991 field season. Study sites included examples of all major natural vegetation types found in the region as well as a number of study plots representing different types of riparian zone restoration treatments being carried out as part of the National Park Service's Glen Creek Restoration Project. Six CSM species were isolated, but two of these--Dictyostelium mucoroides and D. sphaerocephalum--were overwhelming dominants. The total number of clones/g of wet soil ranged from 0 to 1203, with an average value of 259 for all 14 study sites. The mean value recorded for the seven natural vegetation study sites (430) was considerably higher than the corresponding value (87) for the seven restoration study sites. Interestingly, D. mucoroides ranked as the dominant species (59-98% of all clones) for all six of the natural vegetation study sites from which CSM were isolated, whereas D. sphaerocephalum was dominant (50-100% of all clones) in the six restoration study plots with CSM present. (Supported in part by funds provided by the National Park Service.)

DONALD R. NASH and THOMAS E. WEAKS, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2501. The bryophytes of Upper Shavers Fork Basin, West Virginia.

One hundred thirty-seven bryophyte species have been identified from Upper Shavers Fork Basin, in the Allegheny Mountain section of West Virginia. Hepaticopsida included twenty-four species from seventeen genera and fourteen families. Bryopsida included one hundred thirteen species from sixty-seven genera and twenty-eight families. Two liverwort species and ten moss species collected are considered rare for this state and Syrhropodon texanus Sull. is tentatively reported as a new species for West Virginia. The study site was divided into eighteen habitat types including varieties of wetlands, forests and areas disturbed by a variety of sources. The habitats each species occurred in is included in the species list. Bazzania trilobata (L.) S. Gray and Hypnum curvifolium Hedw. occurred in the greatest number of habitat types.

ROGER G. SEEBER, Jr., Dept. of Biology and Chemistry, West Liberty State College, West Liberty WV 26074 Stem Healing of Razor Cuts In six Host plants of the *Enchenopa* Complex.

Insect eggs inserted into woody stems are at risk of being killed by endogenous plant wounding responses. *Enchenopa* (Two Spotted Treehopper) are a complex of insects

which overwinter their eggs under the bark of six different host plants. This strategy may produce negative results if the eggs are overgrown by the stems. To understand these plant responses without insect eggs, stems of the six host genera were slit to the wood with a razor to simulate the ovipositional wound. Callus proliferated into the wound gap of all stems from the cambial zone and secondary phloem but not from the secondary xylem. Callus from the flanks of the gap of the non-leguminous stems merged and filled the gap. The new vascular cambium, which became continuous with the intact vascular cambium, bridged the gap and eventually produced normal secondary xylem and phloem. In contrast, in two legume genera, production of a persistent wound periderm on the periphery of the callus from each flank of the wound gap prevented the merger of their flanking calli and the filling of the wound gap. Although normal vascular cambium and normal secondary xylem and phloem were made in the wound gap, these tissues did not bridge the gap.

The tissues effected by the wound, reacted similarly in both the non-leguminous and leguminous plants, however the mechanisms for closing the gap in these two plant groups varies slightly. The former, floods the gap with tissue and the latter, squeezes the hole closed through lateral callus pressure.

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, TARA DUBEY, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, and ROSEANN DENSMORE, National Park Service, P.O. Box 9, Denali National Park, Alaska 99755. Aquatic fungi in streams of the Kantishna Hills, Denali National Park and Preserve, Alaska.

The aquatic Hyphomycetes occurring in five streams located in the Kantishna Hills of Denali National Park and Preserve in central Alaska were sampled using membrane filtration of stream water. Two other sampling methods (direct examination of organic debris collected from the stream and "baiting" with various types of organic materials) were used to characterize the total assemblage of aquatic fungi present in one stream. In addition, some preliminary data were obtained for the fungi associated with decaying plant material in seepage pools located in areas of subarctic alpine tundra. The total number of Hyphomycetes recorded from all five streams was 48, whereas the number of taxa found in a particular stream ranged from 15 to 23. The number of conidia filtered from stream water ranged from 1865 to 16690 per 1000 ml. The assemblage of aquatic fungi present in the one stream (Glen Creek) sampled more intensively included at least 35 taxa (18 Phycomycetes [chytridiaceous fungi and water molds], 5 non-Ingoldian Hyphomycetes, and 12 Ingoldian Hyphomycetes). Twenty-four taxa were isolated from decaying plant material collected from seepage pools located in areas of subarctic alpine tundra. (Supported in part by funds provided by the National Park Service).

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, CAROLYN J. McQUATTIE, USDA Forest Service, Northeastern Forest Experiment Station, Delaware, Ohio 43015, PAMELA J. EDWARDS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287, and SUSAN M. STUCLAR, Dept. of Botany and Micro-biology, Oklahoma State University, Stillwater, Oklahoma 74078.

The effect of stream pH on the distribution, ecology, and ultra-structure of *Scapania nemorea*.

Scapania nemorea (L.) Grolle, a leafy liverwort, is commonly the dominant bryophyte present in West Virginia mountain streams. In an ongoing study of the possible effect of acidification on bryophyte community structure, we sampled the bryophyte communities associated with a number of streams located on or near the Fernow Experimental Forest in Tucker County, West Virginia. Our data indicate that the relative importance of *S. nemorea* is strongly correlated with pH. In general, the species becomes increasingly dominant with decreasing pH. However, *S. nemorea* is absent from alkaline streams (pH >7.0) and from streams that are extremely acidic (pH <3.2). Transplant experiments carried out during the 1991 field season indicate that *S. nemorea* can survive under these low pH conditions, but a number of changes in cell ultrastructure (as revealed by examination with TEM) are evident. Cell walls of *S. nemorea* collected from a highly acidic stream (pH = 3.9) were thin, mottled and densely stained compared with cell walls of material from a less acidic stream (pH = 5.5). A deterioration in chloroplast structure and other cellular organelles was also observed in material from the highly acidic stream. *Scapania nemorea* transplanted from the less acidic stream (pH 5.5) to an extremely acidic stream (pH 3.2) showed a similar (or less severe) breakdown in cell wall structure and disruption of cellular contents after a period of approximately three months. However, since the most acidic streams included in our study are impacted by acid mine drainage and have high sulfate and/or aluminum levels, low pH may not be the only factor responsible for the observed changes. (Supported in part by funds provided by the USDA Forest Service.)

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University, Huntington, West Virginia 25755.
Bryophyte Communities of Greenbottom Swamp in
Cabell and Mason Counties, West Virginia.

Bryophytes were compared from seven distinct plant communities comprising the major communities of Greenbottom Swamp, a wetland located within the floodplain of the Ohio River. A total of five 50 m transects per plant community were located at intervals of 25 m from the center of each study area. Tree bole communities were sampled at 1 m and at ground level on N, S, E, and W faces. Totals of eighteen moss and three hepatic species occur within Greenbottom Swamp. Heterogeneity and equitability indices indicate that *Salix nigra*-*Acer saccharinum* dominated communities occupying dryer sites have the highest bryophyte species diversity of the seven community types. Dominant mosses occurring on the ground include both acrocarpous and mat forming pleurocarpous species. Dominant mosses are *Amblystegium* spp. and *Leptodictyum* spp. *Amblystegium* spp. are small plants that occur in loose mats in wet habitats. The genus *Leptodictyum* includes a highly variable complex of species many of which are aquatic. The dominant hepatic of the study area is *Riccia fluitans* L. This small hydrophytic species is uncommon in West Virginia.

KEVIN L. WILLISON, MARCIA A. HARRISON, Dept. of
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Huntington, WV 25755

LIGHT-REGULATED ETHYLENE PRODUCTION DURING
GRAVITROPISM IN PLANT STEMS

We investigated the interaction of an environmental stimulus (light) with a physiological response (ethylene production) on upward gravitropic bending in pea stems. In dark-grown pea seedlings, a red light treatment prior to gravistimulation shifts the normal gradual locus of curvature to a more restricted zone resulting in a sharp angle along the stem. Addition of ethylene to red-pretreated seedlings changes this pattern of curvature to resemble that of dark-grown controls. Thus, red light may alter the pattern of gravitropic curvature by changing ethylene production in the plant stem. Red light may cause this change by reducing the amount of ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), in the plant tissue. The following biosynthetic pathway has been established for most plant tissues: methionine \rightarrow S-adenosylmethionine (SAM) \rightarrow ACC \rightarrow ethylene. The level of ACC in plant tissue may be reduced either by inhibition of ACC synthase or metabolism to the malonyl conjugate (M-ACC). For this study, we compared tissue ACC and M-ACC levels in dark-grown and red light-pretreated pea stems before gravistimulation and at 30 min intervals thereafter. Stem segments were homogenized in 80% ethanol and centrifuged at 10,000 x g. ACC level was analyzed by reacting the extract with NaOCl in the presence of Hg to liberate ethylene. Since all ACC is broken down to ethylene by this procedure, then the amount of liberated ethylene can be used to quantify ACC. Ethylene was measured on a gas chromatograph equipped with a flame ionization detector. M-ACC level was measured after hydrolysis of the ACC extracts with boiling 6 N HCl for 1 hour and analyzed for total ACC. In dark-grown stems, the ACC level did not change significantly during gravitropic curvature. However, the level of M-ACC was reduced after gravistimulation. Preliminary results indicate that red irradiation stimulates the conversion from ACC to M-ACC in pea stems. Additionally, changes in the ACC and M-ACC levels during gravitropic curvature in red-pretreated seedlings will be presented.

KAREN M. WISE

Department of Biology
Wheeling Jesuit College
Wheeling, WV 26003. Response of
Juncus effusus to off-road vehicle
disturbance in wetlands of Northern
Canaan Valley, West Virginia.

This study was undertaken to measure the response of Juncus effusus to off-road vehicles. J. effusus, an emergent wetland plant, was studied in Northern Canaan Valley, West Virginia. Samples of J. effusus were collected in disturbed and relatively undisturbed reference sites. Length of 150 J. effusus stems in a disturbed site were compared to 150 stem lengths collected in a reference area. Stem lengths were greater in the undisturbed area than in the area disturbed by off-road vehicles. Samples of the above ground portion of J. effusus were dried and weighed. Biomass at reference sites was greater than biomass at the most severely disturbed sites. However, biomass of J. effusus was greater in some areas that had been lightly disturbed (up to 100g/m²), compared to undisturbed or

severely disturbed areas (40 and 10g/m² respectively). J. effusus appears to be more resistant to off-road vehicle disturbance than other plants, and is possibly more resilient than other species by claiming nutrients in exposed soils.

Chemistry

KIMBERLY M. ANTINORO, Dept. of
Chemistry, West Virginia University,
Morgantown, West Virginia 26506.
Carbamazepine: Its Use in the Treatment
of Epilepsy.

Today, carbamazepine has become one of the leading drug treatments for epileptic patients with complex partial seizures and tonic-clonic (grand mal) convulsions. Although the mechanism of action of carbamazepine remains unknown, the biochemical mechanism can be postulated based on appropriate laboratory experiments. The results of these experiments and their implications for the bioactions of carbamazepine will be discussed.

JACQUELINE BENNETT, Dept. of Chemistry,
West Virginia University, Morgantown,
West Virginia 26506. Polymer Composites

A composite is a material in which two or more distinct materials having distinct properties are combined to yield a new material with properties greater than those of its constituents. These increased properties include strength, stiffeners, and toughness. Other composite properties such as resistance to corrosion, creep, fatigue, temperature, and moisture are also important considerations when choosing a composite material for a specific application. Of particular interest are fiber polymer composites, in which the fiber provides the composite with strength and resistance to deformation. The matrix maintains the spacing and location of the fibers with respect to one another and also protects the fibers from surface damage. The properties of a composite vary depending upon the properties of its constituents. The effects of various combinations of fibers and matrix on the overall composite property as well as their application will be discussed.

JOHN T. BROWN and PLATO A. MAGRIOTIS,
Dept. of Chemistry, West Virginia
University, Morgantown, West Virginia
26506. Stereo- and Regiospecific
Formation of Vinylstannanes and Their
Application to Synthesis.

Vinylstannanes have been found to be highly useful synthetic intermediates. Addition of a trialkyltin hydride to an acetylene is the easiest method of vinylstannane formation, but this method usually leads to the formation of stereo- and regioisomers. Given the need for highly stereo- and regiospecific vinylstannanes in natural product synthesis, the ability of thio-substituted alkynes to yield such species will be discussed.

TRACI EDDY, Dept. of Chemistry, West
Virginia University, Morgantown, West
Virginia 26506. Treatments of
Alzheimer's Disease.

Although a treatment has not yet been found for Alzheimer's disease, much research has been focused on the demonstrated alterations in the brains of Alzheimer patients. The hallmark of Alzheimers' disease is marked depletion of the cholinergic neurotransmitter acetylcholine. Because the cholinergic system has been shown to play a major role in learning and memory, much research has been directed toward enhancing the synthesis and release of acetylcholine. The results of experiments relating the role of acetylcholine to Alzheimer's disease will be presented, along with potential treatments of this increasingly important disease.

PRISCILLA R. HIGGINS
Department of Chemistry
West Liberty State College
West Liberty, WV 26074

The Analysis of Contaminates in Water

Much has been said about contaminates in rainwater and groundwater especially the concern about WTI (toxic waste incinerator being built) emissions once in operation. This concern was the reason for doing such project. This project is geared toward the analysis of rainwater, groundwater, and the soil near the groundwater.

The preparation and procedures used are EPA approved from the text, Standard Methods, 15th ed., 1980. All the samples will be collected in plastic bottles to avoid contamination with any metals, which would give inappropriate results.

Determination of total metals (calcium, copper, iron, lead, cadmium, and arsenic) are being analyzed by an air-acetylene flame atomic absorption (p 152). The Dithizone method will be used for analyzing mercury with the use of the DMS 100s UV-spectrophotometer (p 217). For fluoride, the Electrode method will be used (p 335). For the test of nitrogen, the Ultraviolet Spectrophotometric Screening method will be used (p 368). Sulfates will be analyzed by using the Turbidimetric method (p 439).

The soil samples for the above analysis will be using the TCLP (Toxic Leaching Procedure). This will consist of 90.00 g of the soil sample with a solution of pH 2 (nitric acid, acetic acid, and deionized water). The samples then will be allowed to shake overnight to thoroughly saturate the soil samples with the solution.

The determination of certain organics will consist of the liquid-liquid extraction method, where the acid and base side will be extracted with methylene chloride and then concentrated down to 2.00 mL. This will then be ran on the liquid chromatograph.

ROBERT HORACEK, Dept. of Chemistry, West Virginia University, Morgantown, West Virginia 26506. Hydrogen Dihalides: An Introduction to Heavy-Light-Heavy (HLH) Collisions.

Since their discovery in 1968, triatomic complexes such as HCl_2^- and HBr_2^- have increased in importance due to their frequent occurrence as reaction intermediates. The potential energies of these intermediates have been studied by a variety of theoretical methods, including BEBO, classical, and semi-classical. In our laboratories, these ions are being examined for their role in understanding chaos near transition state structures. The importance of these results for chemical reactions will be discussed.

ROLF MARTIN, Department of Chemistry, Box 168, POB 1000, West Virginia State College, Institute, WV 25112-1000. Acid Rain and Chemical Emissions: Should State Science Academies Respond When Public Scientific Controversies Fester And Opposing Positions Are Not Submitted To Scientific Review?

To explore this question, two current controversies will be examined: (1) Is acid rain a serious threat to our lakes and streams? (2) Do chemical emissions represent a significant health threat to Kanawha Valley residents? The CBS show "60 Minutes" twice broadcast an episode on acid rain which included assertions that "the acid rain problem is so small that it's hard to see" and that Congress is wasting "about \$4,000,000,000 a lake" for a politically motivated, scientifically unjustified cleanup. The show ignored evidence that acid rain is causing fish populations to decline dramatically -- and in some cases to disappear completely -- in a number of West Virginia,

Virginia and Pennsylvania streams and rivers. After the first airing, a number of scientists and EPA officials called and wrote to the producer to explain how seriously incomplete and misleading the discussion appeared. Despite these protests, the show was scheduled for rebroadcasting once again during the Summer. When scientists learned of the planned rebroadcast, the senior producer was called and a letter explaining the nature of the problem was hand-delivered to his office in New York City. This letter included the names and phone numbers of WVDNR personnel who could discuss and verify evidence that acid rain is indeed a very serious threat to many WV fish populations. The WVDNR representatives were never contacted and the show was rebroadcast with only minor ammendment several weeks later. During the second controversy to be considered, Prof. Basudeb DasSarma (Chemistry Department, WV State College) repeatedly criticized a NICS-Harvard study of chemical health risks in the Kanawha Valley in letters to government officials and in articles published in the Charleston Gazette. His main point was that published estimates of local health risks have been seriously understated and investigations have been unproductive and wasteful, because the most potent carcinogens -- accounting for over 90% of the local cancer risk -- were neither measured nor included in risk calculations. At one point, Prof. DasSarma was called "misguided" and "ill-informed" by the chief investigator for the Harvard research team. When the Kanawha Valley Section of the American Chemical Society and an ad hoc symposium organizing committee decided to conduct a scientific review of the issue, Prof. DasSarma agreed, but the chief Harvard investigator declined, to submit a paper for scientific review. Laypersons who see experts contradicting each other in public may well conclude that science is an unproductive mode of thought, incapable of answering even the technical, chemical questions that our society faces. State academies may reduce this problem with relatively little effort by inviting opponents to submit their positions to scientific review in existing publications, or may arrange panel discussions of key issues at annual conferences, with the understanding that position papers be formally reviewed and published afterward. If academies do not respond to local or regional "miscarriages of science" then who will? Unfortunately respondents may be organizations or individuals who have neither the expertise nor the objectivity to offer convincing explanations, and the issues will fester.

L.R. Meadows, D.G. Morris, C.K. Pan, F.L. King, Dept. of Chemistry, West Virginia University, Morgantown, West Virginia 26506. Characterization of Argon/Methane Plasma Chemistry Relevant to the Deposition of Diamond Films.

Mass spectrometry and optical spectroscopy have been employed in the characterization of an argon/methane glow discharge plasma. Investigations were conducted into the relationship between the plasma chemistry and the percent dilution of a CH_4/H_2 mixture with argon. Results from these investigations into the role of argon dilution as determined from plasma temperature dependant changes in the ionic population of the plasma will be discussed.

SCOTT ROSENCRANCE, Dept. of Chemistry, Fairmont State College, Fairmont, West Virginia 26554 and JAMES COLEMAN Dept. of Chemistry, Fairmont State College, Fairmont, West Virginia 26554. An Investigation Into the Mercury (II) Catalyzed Dissolution of Aluminum

Mercury (II) displays remarkable catalytic activity in the dissolution of aluminum. Reaction profile studies clearly show that there is an optimum catalytic concentration which is temperature dependent. Atomic absorption analysis suggests that the reaction system partially stabilizes the presence of Mercury (I) and/or metallic mercury in 3 M nitric acid. Calculations from rate studies at various temperatures yield a low activation energy for the system.

STEPHANIE A. SCHWER, Dept. of Chemistry, West Virginia University, Morgantown, West Virginia 26506. Structural Characteristics of Copper (II) Nitrate.

Cupric nitrate, $\text{Cu}(\text{NO}_3)_2$, is a common compound whose structure is of current interest. Investigations using a variety of methods were used to characterize the structure of the nitrogen moieties surrounding the copper ion. In this talk, the results for cupric nitrate obtained by mass spectroscopy, electron diffraction, x-ray diffraction, EPR spectroscopy, and infrared-Raman spectroscopy will be discussed.

RONALD A. SWEARINGEN, Dept. of Chemistry, West Virginia University, Morgantown, West Virginia 26506. Synthesis and Doping of Buckminsterfullerene to Produce Superconducting Lattices

Since it was first discovered in 1985, Buckminsterfullerene has become the subject of a vast amount of research and theoretical interest. The discovery of the superconductive properties of the alkali-doped C_{60} molecule has led to critical temperatures in excess of 40°K . The synthesis and stereo-specific relationships of these fullerenes will be discussed with an emphasis on the K_3C_{60} single phase structure as well as the Rb-doped-Thallium doped fullerene.

KENNETH B. WILBERG, JOSEF MICHL, RICHARD MORRISON, and DAVID MCANALLEN, Dept. of Chemistry, West Virginia University, Morgantown, West Virginia 26506. The Synthesis of Propellane and, from it, the Synthesis and Uses of Bicyclopentane Oligomers.

Propellane is a rather unstable compound first synthesized from dibromocarbenes only several years ago. It is of much importance in synthesizing bicyclopentane oligomers, which have many practical applications in today's world. These oligomers are building blocks of molecular grids which have possible memory storage applications. The bicyclopentane oligomers are also molecular spacers and they demonstrate electron transfer properties. The synthesis and attempts to test the capabilities of these molecules in these applications will be discussed.

Ecology

MARY BETH ADAMS, USDA Forest Service, Parsons, West Virginia 26287 and KIM G. MATTSON, University of Idaho, Corvallis, Oregon 97331. Effects of Nitrogen on Carbon Turnover in Forests

To test the hypothesis that nitrogen additions suppress belowground carbon cycling rates, soil CO₂ efflux was measured on two forested watersheds on the Fernow Experimental Forest, West Virginia. One of the watersheds had received experimental additions of nitrogen equalling approximately 3X ambient deposition levels, as part of an artificial watershed acidification project. Carbon dioxide efflux also was measured on an adjacent control watershed. Detrital carbon standing stocks and fine root masses had been assessed previously on these watersheds. Carbon dioxide evolution was measured by the static technique of adsorption in NaOH at five sampling dates from June 20 to July 13, 1991. Carbon dioxide efflux was chosen for the measure of carbon dynamics since it is judged to be the most sensitive measure of short-term changes in rates of carbon cycling in soils. Watershed differences in CO₂ efflux were highly significant, with efflux rates from the treated watershed significantly lower than those of the control (an 8% reduction). Soil temperatures were significantly lower and soil moistures significantly higher on the treatment watershed. Soil CO₂ efflux also was measured on two older (70-80 yr) watersheds and was found to be significantly different between these two watersheds, which suggests that the watershed-to-watershed variability may be high. This research will be continued this summer to further refine the hypothesis and conclusions.

JEFFREY E. BAILEY and THOMAS K. PAULEY,
Department of Biological Sciences, Marshall
University, Huntington, West Virginia 25755.
Ecological Aspects of the Cumberland Plateau
Salamander, *Plethodon kentucki* Mittleman
(1951), in West Virginia.

An ecological study of the Cumberland Plateau Salamander, *Plethodon kentucki*, was conducted in southwestern West Virginia during 1990-1991. Results of studies on range and distribution in West Virginia indicated that populations of *P. kentucki* occur in at least 13 counties, all in the southwestern portion state. The New-Kanawha River system appears to be the boundary line for the northeastern extent of this salamander. A population of *P. kentucki* studied at Beech Fork State Park in Wayne County, West Virginia, appeared to be extremely seasonal. Approximately 70% of *P. kentucki* observed during 15 months of study (June 1990 - August 1991) were found in March and April of 1991. Seasonal activity was

significantly correlated with soil moisture, while air and soil temperature, air relative humidity, and soil pH were not significantly correlated with seasonal activity. Activity of salamanders during a 24 hour period peaked between the hours of 9PM and 10PM EST. Results of aspect studies revealed that salamander abundance is greater on west-facing (44.8% of salamanders observed) slopes than on southwest- (31.0%) and northwest-facing slopes (14.6%). Rocks were utilized as cover objects (47.9%) more frequently than roots (27.1%) or logs (4.2%). Salamanders found within leaf litter, or on its surface constituted 20.8% of the total observations.

DANIEL W. CHAFFIN, DONALD R. NASH, MICHAEL K. NOWLIN and THOMAS E. WEAKS. Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2510.
Distribution patterns of hepatic species of a wooded ravine.

The cover of hepatic species was estimated in six different microhabitat types occurring within a wooded ravine located in Wayne County West Virginia. Hepatic communities were compared using coverage, species diversity, prominence index, and cluster analysis. Few hepatic species of the study area are restricted to a given microhabitat. However, different microhabitats tend to bear different hepatic communities. Distribution patterns can be attributed in major part to the relationship of growth form to water proximity. Major changes in prominence values of a number of hepatics occurred in the vicinity of certain elevations, when the site was viewed as a whole. Zonation was rather distinct, primarily resulting for the absence of moisture gradients and available substrates that restricted hepatic colonization.

RAYMOND C. DERK AND JOSEPH P. CALABRESE,
Department of Environmental Microbiology, West Virginia University, Morgantown, WV. Application of the Methylcarbamate Gene Probe to Indicate Potential Carbofuran Degradation in Anaerobic Sediments.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benofurayl-N-methylcarbamate) is a broad spectrum carbamate insecticide/nematicide employed for pest control in agricultural production of several crops including corn, cotton, peanuts, apples, and peaches. Carbofuran efficacy relies on a residual activity of 4 to 8 weeks following application. Aerobic microbial activity has been implicated in reduction of this activity to 1 to 2 weeks. However, recent concerns over groundwater contamination by highly soluble, highly mobile pesticides, such as carbofuran, lead us to investigate the effect of anaerobic microbial activity on the breakdown

of this compound. Studies were initiated as a result of hybridization of the methylcarbamate degradation (*mcd*) gene probe at a 10kb region of total bacterial DNA extracted from anaerobic sediments collected from constructed *Typha* wetlands, which have no previous history of carbofuran application. Sediment microcosm samples were enriched with 200 ppm carbofuran, incubated in an anaerobic chamber, and monitored for disappearance of pesticide with time. After 90 days incubation, we detected up to an 80% reduction in carbofuran in the live samples when compared to killed controls. A 77% maximum increase in carbofuran-phenol, a hydrolysis by-product of carbofuran, was also detected in the samples. We have initiated a second phase of the study which includes enrichment of the microcosms a second time with carbofuran (200 ppm) and isolation of bacteria capable of utilizing the compound as a sole source of carbon and/or nitrogen. Data from degradation studies, isolation studies, and gene probe hybridization will be presented.

FRANK S. GILLIAM, Dept. of Biological Sciences,
Marshall University, Huntington, WV 25755 and
MARY BETH ADAMS, NE For. Exp. Sta., Parsons,
WV 26287. Stand structure and species composition of
montane hardwood forests of West Virginia.

A variety of factors influence the structure and composition of high-elevation forests. This study examined stand structure and species composition in three watersheds of the Fernow Experimental Forest, Parsons, WV, focusing on elevation relationships within watersheds and stand age/history relationships between watersheds. These watersheds were WS4 (control; 81 yr), WS3 (cut; 21 yr), and WS7 (cut/herbicide; 21 yr). A total of 40 circular 0.04-ha plots were established, 15 each in WS4 and WS3, 10 in WS7. All woody stems ≥ 2.5 cm diameter at breast height (DBH) were identified, tallied, and measured for DBH. WS3 and WS7 were similar for many variables, contrasting sharply with WS4. These variables included species richness (25 and 23 vs. 37 species/ha, for WS3 and WS7 vs. WS4, respectively), stem density (2413 and 1835 vs. 945 stems/ha), and total basal area (24.0 and 19.1 vs. 38.2 m²/ha). Total basal area and stem density decreased with elevation on WS4, in contrast to general patterns of increase for WS3 and WS7. Dominant species were *Acer saccharum* (sugar maple)/*Quercus rubra* (northern red oak) for WS4 and *Prunus serotina* (black cherry)/*A. saccharum* for both WS3 and WS7, although black cherry was nearly the sole dominant for WS3. Tree species basal area correlations and size-class distributions suggest a species replacement of black cherry by sugar maple through secondary succession. These results indicate that 1) elevation, stand age, and stand history greatly influence structure and composition of these forests, 2) the successional sequence at this site shifts from black cherry dominance in young stands to sugar maple/red oak dominance in mature stands, and 3) the herbicide treatment may have accelerated this process on WS7.

TOM JONES and DONALD TARTER. Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Spring season foraging patterns of fishes in the Green Bottom Wildlife Management Area, West Virginia, in relationship to macrobenthos and planktonic community structures.

The Green Bottom Wildlife Management Area is located 16 miles north-east of Huntington, West Virginia, along the Cabell and Mason county line. Approximately 60ha of wetlands occurs on this mitigation property. A limited ichthyofauna exists in this wetland due to its fluctuating temperatures and dissolved oxygen levels. Studies comparing prey community structure to predator feeding patterns in wetlands are limited in the literature. Fishes were collected monthly (February to May), and their stomach contents analyzed (percent frequency occurrence and percent total volume). Plankton samples were taken and macrobenthos were collected using a square meter sampling technique. Four weeks prior to collection, nine stations were selected and Hester-Dendy multiplates (27) were set to allow colonization of macrobenthos. Prey community structure shifts were analyzed using ANOVA. Also, prey taxon densities were compared to stomach contents. These results will be discussed in relation to species-specific foraging patterns.

AMY C. KOKESH and DAVID L. EDENS, Dept. of Biology, and ROLF J. MARTIN, Dept. of Chemistry, West Virginia State College, Institute, West Virginia 25112-1000. Analysis of plant and macroinvertebrate communities and toxic metal concentrations indicates no biological amelioration of acid mine drainage near Mt. Storm Lake, WV.

An abandoned surface mine, occupying 128 hectares west of the Allegheny Front and within the Mt. Storm Lake watershed, Grant County, WV, was investigated to determine the possible extent of any natural, biological amelioration of acid mine drainage (AMD) within the site, and to identify those vascular plant species associated with acidity reduction and/or removal of toxic metals from soils and/or influent and effluent streams. Vegetational data also were collected to determine plant communities of the site, and the extent of ecological succession which has occurred since abandonment, approximately twenty years ago. Influent and effluent streams were sampled for aquatic macroinvertebrates as biological indicators of water quality, and recordings made of physiochemical parameters, e.g. pH, temperature and dissolved oxygen. Initial analyses of streams and soils were conducted to determine concentration levels of aluminum, antimony, arsenic, cadmium, cobalt, copper, chromium, iron, lead, manganese, molybdenum, nickel, selenium, silver, thallium, vanadium and zinc. Primary streams were then investigated to determine concentration levels of aluminum, copper, iron and manganese, as well as acidity, hardness and sulfates. Complete analyses of water and soil samples indicated no evidence of amelioration of AMD within the study area, and no correlation of plant species with acidity reduction and/or removal of toxic metals from influent/effluent streams, beaver ponds or cattail marshes. Given the age of the site and the domination of

pioneer plant species, the site appears to be severely lagging in progression through "typical" seral stages of secondary succession. Low macroinvertebrate species diversity and equitability biologically confirmed the poor water quality revealed through chemical analyses, ranging from streams supporting no aquatic species/genera to those supporting species/genera capable of tolerating high concentrations of iron and manganese.

GLENN R. MILLS and THOMAS K. PAULEY,
Dept. of Biological Sciences, Marshall
University, Huntington, WV 25755. Comparative
analysis of stomach contents between sympatric
populations of *Desmognathus quadramaculatus*
(Holbrook) and *Desmognathus monticola* Dunn in
West Virginia.

Dietary competition and influence of head width on prey size and selection in different age classes of two desmognathid salamanders were studied. *Desmognathus quadramaculatus* reaches the most northern edge of its range in Fayette County, West Virginia, and it is currently listed as a species of concern by the West Virginia Division of Natural Resources. *Desmognathus quadramaculatus* is the most aquatic desmognathid found in West Virginia and has rarely been found far from streams. *Desmognathus monticola* also is aquatic, however, it occasionally is found on land in moist areas along streams. The study consisted of 94 specimens of *D. quadramaculatus* and 39 *D. monticola* that were collected at the same time and from the same streams. Stomach contents showed competition for many types of prey when the two salamanders were of equal size, however, *D. quadramaculatus* adults were able to utilize larger prey resource probably because of their larger size and proportionally larger head width.

MATTHEW MONTANI and THOMAS K. PAULEY,
Department of Biological Sciences, Marshall
University, Huntington, WV 25755. Status of
Eurycea bislineata (Green) in West Virginia.

Previous to 1987, *Eurycea b. bislineata* (northern two-lined salamander) was considered to be the only representative of the *E. bislineata* group to occur in West Virginia. However, electrophoretic work by Jacobs (1987) showed that two species, *E. bislineata* and *E. cerrigera*, are found in West Virginia. Jacobs indicated that *E. bislineata* occurred in approximately the northern one-third of the state and *E. cirrigera* occurred in the southern two-thirds. Morphologically, Jacobs showed that the two species can be distinguished by costal groove counts, i.e. *E. bislineata* has 15 to 16 grooves and *E. cirrigera* has 14. Based on costal groove numbers, we examined 198 specimens representing 49 counties from the West Virginia Biological Survey at Marshall University to

determine the ranges in the state. We found that 83.7% of *E. cirrigera* (i.e., specimens with 14 grooves) were found south of Kanawha River and west of New River and 98.7% of *E. bislineata* (15-16 grooves) came from north of Kanawha River and east of New River. It appears, therefore, that the Kanawha-New River system is a natural barrier separating ranges of these two species in West Virginia.

THOMAS K. PAULEY, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Report on amphibian and reptile roadkills in West Virginia.

Records were maintained from 1987 through 1991 on amphibians and reptiles observed dead on roads (DOR) in West Virginia. During these 5 years, I traveled 124,134 miles and observed 347 specimens. While many amphibians are observed dead on highways during night surveys in areas of spring migrations, only 5 specimens were observed during this study. Most amphibian species decay faster than reptiles and are consumed by scavengers shortly after they are killed. Reptiles observed on roads included: *Terrapene c. carolina* (eastern box turtle) - 246 (70.9%); *Thamnophis s. sirtalis* (eastern garter snake) - 37 (10.7%); *Elaphe o. obsoleta* (black rat snake) - 27 (7.8%); *Lampropeltis t. triangulum* (eastern milk snake) - 8 (2.3%); *Chelydra s. serpentina* (common snapping turtle) - 5 (1.4%); *Nerodia s. sipedon* (northern water snake) - 4 (1.2%); *Opheodrys v. vernalis* (smooth green snake) - 4 (1.2%); *Agkistrodon contortrix mokasen* (northern copperhead) - 4 (1.2%); *Coluber c. constrictor* (black racer) - 3 (0.9%); *Lampropeltis getulus niger* - 2 (0.6%); *Diadophis punctatus edwardsii* - 2 (0.6%); *Opheodrys aestivus* (rough green snake) - 1 (0.3%); *Heterodon platirhinos* - 1 (0.3%). Amphibians observed included: *Bufo a. americanus* (eastern American toad) - 1 (0.3%); *Rana sylvatica* (wood frog) - 1 (0.3%); *Notophthalmus v. viridescens* - 1 (0.3%).

RONALD PRESTON, United States Environmental Protection Agency, Wheeling, West Virginia 26003. The use of biological criteria in environmental regulations.

The USEPA National Biocriteria Program was initiated in response to the mandate of the Clean Water Act to restore and maintain "...the chemical, physical and biological integrity of the Nation's waters." Historically, parameters in the first two of these categories have been emphasized in the measurement and management of our surface waters. It is only recently that sufficient advances and refinements have been made in the biological arena to satisfy managers and sci-

entists of the broad application of this approach to water resource protection and management.

In acknowledgment of these advances, the Agency established the biocriteria Program to add biological methods to the existing physical and chemical approaches to surface water measurement and management. National program guidance was prepared for the development of biological criteria as part of state water quality standards.

States will be expected to adopt "narrative criteria" for their surface waters by the end of the 1991-1993 triennium and the Biocriteria Program has begun preparation of technical guidance material to aid in this process. Technical publications for streams, lakes, rivers, wetlands, estuaries and marine waters will be prepared and distributed.

CHRISTOPHER T. RUHLAND AND BEN M. STOUT III
Dept. of Biology, Wheeling Jesuit College,
Wheeling, West Virginia 26003. Case
Building Efficiency of Pycnopsyche sp.:
Early vs. Late Successional Leaves.

Caddisfly larvae (Pycnopsyche sp.) were collected from forest streams in mid-November at an early instar stage. Early successional leaves (sassafras, dogwood, redbud, sumac) and late successional leaves (beech, hickory, white oak, red oak) were softened in an aquatic environment fourteen days prior to exposure to larvae. Individual larvae were removed from their original case and placed with leaves in 0.5-liter chambers at 10°C. Each leaf species was replicated using five larvae. Case lengths were measured using digital image analysis. Original cases were measured, and new cases were measured at regular intervals up to 456 hours. Penetrometry was used to compare leaf rigidity, and in general early successional leaves were less rigid than late successional leaves. Case completion was generally greatest for early vs. late successional leaf species, and differences were significant 6 to 72 hours in the study. However, after 120 hours there were no significant differences when comparing early versus late successional leaves. This was attributed mostly to sassafras, an early successional species for which case building was relatively slow, and hickory, a late successional species providing relatively fast case completion. Sassafras was less penetrable than other early successional species and hickory was more penetrable than other late successional species. A significant negative correlation was found between casebuilding and penetrence, and up to 83% of the variation in casebuilding efficiency could be explained by leaf penetrence. Larger, more regular cases were built from early successional leaves, whereas late successional leaves yielded irregular shaped cases.

STACI D. SMITH, NICOLE L. TURRILL,
FRANK S. GILLIAM, Dept. Biol. Sciences,
Marshall Univ., Huntington WV 25755
and MARY BETH ADAMS NE For. Exp.
Sta., Parsons, WV 26837. Herbaceous
layer species composition, richness,
and diversity of a southern Appalachian
mixed hardwood forest.

Study of the herbaceous layer is essential for a more complete understanding of a forest ecosystem. The objective of this study was to describe the species diversity and richness of the Fernow Experimental Forest, Parsons, WV. Watersheds were selected on the basis of stand age and history (WS3-21 yr, clearcut; WS4-81 yr, control; and WS7-21 yr, clearcut and herbicide). Each watershed had 15 circular sample plots with a radius of 11.3m (0.04 ha) except for WS7 (10 plots). Within each plot, 10 randomly located subplots (1m²) were used for herb sampling. All vascular plants <1m in height were identified to species and estimated for cover (%). Species richness was 65 species/ha, 68 species/ha, and 103 species/ha for WS3, WS4, and WS7, respectively. Species diversity (H') was determined on the basis of cover. Average diversity was 1.71, 1.61, and 1.85 for WS3, WS4, and WS7, respectively. Diversity was negatively correlated with elevation in WS3 and WS4; no relationship was found in WS7. Other variables, such as herb layer cover or tree basal area, were not correlated with herb layer diversity in any watershed. Several species (e.g., Laportea canadensis, Viola sp., and Acer pensylvanicum) were dominant in both WS3 and WS4. In contrast, WS7 was dominated by ferns (Dryopteris marginalis, and Polystichum acrostichoides). Species with highest cover (Laportea canadensis and Viola sp.) had an affinity for high elevations in WS3 and WS4. The dominant ferns in WS7 showed an affinity for middle elevations. These data suggest that 1) herb layer species richness and diversity do not increase through succession in these forests; 2) species diversity response to elevation is a function of response of individual herb layer species to gradient conditions and 3) herbicide treatment may have influenced herb layer succession on WS7.

CRAIG W. STIHLER, Div. of Natural Resources, Endangered Species Program, Elkins, WV 26241 and VIRGIL BRACK, JR., 3D/Environmental Services, Inc., Cincinnati, OH 45233. A survey of hibernating bats in Hellhole Cave, Pendleton County, West Virginia.

On 16 February 1991, three survey crews spent approximately 99 person-hours conducting a mid-winter bat survey of Hellhole Cave, Pendleton County,

WV, the most significant bat hibernaculum in West Virginia. A total of 61,791 bats of 8 species were tallied. Species noted were Myotis lucifugus (49,707) M. grisescens (2), M. spetentrionalis (2), M. sodalis (5,470), M. leibii (2), Pipistrellus subflavus (417), Eptesicus fuscus (3), and Plecotus townsendii (6,188). These populations represent the largest known colony of P. townsendii, the largest known colonies of M. lucifugus and M. sodalis in West Virginia, and the first recent record of M. grisescens in West Virginia. Also observed in the cave was one fresh dead specimen of Lasiurus borealis.

BEN M. STOUT III, and KATHY K. STOUT.
Department of Biology, Wheeling Jesuit
College, Wheeling, WV, 26003, USA.
Video image analysis methods for remote
sensing of off road vehicle damage in
northern Canaan Valley, West Virginia.

Upland and wetland plant communities in northern Canaan Valley, West Virginia, have received considerable damage from unrestricted off road vehicle (ORV) use. We used remote sensing followed by vegetation sampling to measure the area and extent of ORV disturbance in fourteen plant communities. Disturbed and reference areas were measured from low-level aerial photographs using a personal computer equipped with a commercially available video imaging system. Area measurements were calibrated from panels placed at 100-meter intervals on the ground, resulting in 2.5% scaling error. Densitometry, or grey-scale analysis, was used to delineating severely disturbed areas based on the frequency of screen pixels along a scale of 256 grey shades. Severely disturbed wetland areas had less than 10% of the plant biomass of relatively undisturbed reference sites, and disturbed soils were closer to the black end of the grey scale than the surrounding vegetated areas. Disturbed upland areas were characterized by light sandy loam soils that were closer to the white end of the grey scale than reference areas. Densitometry was also an excellent tool for measuring percent plant cover from photographs of vegetation samples taken during ground surveys.

Mark C. Turner, Department of Environmental
Science/Zoology, Prescott College, Prescott
Arizona 86301 and Thomas K. Pauley, Department
of Biological Sciences, Marshall University,
Huntington, WV 25755. An ecological study of
Desmognathus quadramaculatus (Holbrook) in
Keeney's Creek, Fayette County, West Virginia.

Three sites on Keeney's Creek, Fayette County were studied from June through August 1991 to determine surface densities, age/size classes, sex ratios, movement patterns, and cover objects of D. quadramaculatus and a sympatric congener D. monticola. Because both species, particularly D. quadramaculatus, are considered to

be excellent fishbait, the three sites were selected to test bait collecting pressure by fisherman. One site was located near the confluence with New River, one was upstream approximately 2.5 miles, and one was located between these two. The upper and middle sites were far enough from New River to minimize collecting pressure from fisherman. Surface densities included 43 D. quadramaculatus and 32 D. monticola in the upper site, one specimen of each species in the lower site, and 47 D. quadramaculatus and 37 D. monticola in the middle site. Size class ratios were approximately 2:1 (adults:juveniles) for both species, and sex ratios were approximately 2:1 (male:female) for both species. Linear distances moved by D. quadramaculatus averaged 3 meters, the greatest distance was 7 m and the shortest distance was 1 m. Cover object sizes ranged from 15.2 to 45.7 cm for both species with an average of 27.9 cm for D. quadramaculatus and 29.0 cm for D. monticola.

NICOLE L. TURRILL and FRANK S. GILLIAM,
Dept. of Biological Sciences, Marshall
University, Huntington, WV 25755, and
MARY BETH ADAMS, NE For. Exp. Sta.,
Parsons, WV 26287. Response of herbaceous
layer vegetation to elevation in a southern
Appalachian hardwood forest.

Herbaceous layer vegetation is potentially sensitive in response to varying gradient conditions such as elevation. The purpose of this study was to examine several aspects of herb layer vegetation of the Fernow Experimental Forest. Selected watersheds [WS3--21 yrs (clearcut); WS4--81 yrs (control); WS7--21 yrs (clearcut/herbicide)] each spanned an 80-m elevational gradient and were composed of mixed hardwood stands. Circular 0.04-ha sample plots were established in each watershed (15 in WS3 and WS4; 10 in WS7). Cover (%) was determined for all species within 10 1-m² sub-plots in each sample plot. Two sub-plots having greatest cover in each sample plot were harvested for biomass. All living woody stems ≥ 2.5 cm diameter breast height (dbh) within the sample plots were measured. Herb layer biomass was significantly ($P < 0.001$) correlated with cover for all watersheds; this regression was used to estimate biomass for non-harvested sub-plots. Mean herb layer cover was 39.7% for WS7 followed by 26.4% for WS4 and 19.3% for WS3. Mean herb layer biomass was 26.4, 13.0, and 11.6 g/m² for WS7, WS3, and WS4, respectively. Herb cover and biomass were positively correlated with elevation for all watersheds. Understory (but not overstory) basal area and density on WS4 were negatively correlated with herb cover and biomass. Overstory (but not understory) basal area on WS3 was positively correlated with herb cover and biomass. On WS7,

overstory basal area and density were negatively correlated with herb biomass. These data suggest that 1) herbaceous vegetation responds to closure of the understory canopy in mature, well-stratified stands, 2) other gradient conditions (e.g., moisture, nutrient concentrations) affect the herb layer in young, even-aged stands, and 3) the herbicide treatment (WS7) has caused long-term (successional) changes in herb layer vegetation.

V. ULRICH, Div. of Plant and Soil Sciences
West Virginia University, Morgantown, WV
26506-6108 and O.A. FINLEY, Star Rt. Box
7, Levels, WV 25431. Long term orchard
ground cover integrity and associated
weeds in three seeded grasses.

Observations of orchard alleys seeded to tall fescue, brome grass and perennial ryegrass found that the latter species declined rapidly and was not an effective orchard alley grass. Brome grass and tall fescue persisted and maintained excellent alley grass orchard floors even after 15 years. Brome grass attracted significantly more deer trespasses than the control or tall fescue. This may have resulted in the introduction and establishment of significant horse nettle and alfalfa populations. The lower frequency of deer trespass and the allelopathic properties of tall fescue apparently reduced the presence of weeds, especially those of horse nettle and alfalfa.

Education/Psychology

B. DASSARMA, Department of Chemistry, West Virginia State College, Institute, West Virginia 25112. Scaled down Integrated experiments to reduce pollution, cost, and risk in general chemistry laboratory.

A small part of experimental chemistry can be introduced to undergraduate students due to the constraints of cost and time involved in laboratory courses. Current awareness on minimizing environmental pollution, the very high cost of laboratory wastes mandated by EPA, and the availability of low cost digital analytical balances have revived scale down (popularly called micro scale) of operations by 1/10 to 1/1000. About 17% of laboratory experiments in organic chemistry in the United States were reported in 1991 at an appreciably reduced scale. Freshman chemistry laboratory at West Virginia State College involves over 1000 student laboratory hours per year. A program is underway to modify general chemistry laboratory experiments by integrating several experiments, where home-made individualized equipment and procedures will be utilized in addition to scaling down the amounts of chemicals used. An integrated scheme will be described where measurements of number in a mole, water of hydration, chemical stoichiometry with laws of constant and multiple proportions can all be combined in sequential steps.

E. RAY GARTON. EnviroCheck, PO Box 200, Barrackville, WV 26559. PRE CONSTRUCTION RADON SOIL GAS MEASUREMENTS AT THE EDUCATION & HEALTH CAREERS BUILDING SITE AT FAIRMONT STATE COLLEGE.

Elevated radon levels were measured in three buildings on the Fairmont State College campus in testing conducted in December 1989 and May 1990. The affected areas have undergone additional testing, diagnostics and mitigation plans are being designed. Because elevated radon levels were well documented on campus EnviroCheck was retained to determine if there was a potential for elevated radon in the new Education & Health Careers Building. Soil gas testing was chosen as the best method to determine if there was a potential for elevated radon in the new building. Based on the foundation plan of the new building (approximately 20,000 square feet) radon gas monitoring wells were placed throughout the foundation plan area. A total of 42 monitoring wells were placed on approximately 400 square foot centers. The wells were constructed of 4 inch diameter PVC and were 40 inches long. The wells range from a depth of 4-26 inches. An E-PERM electret ion chamber radon detector was placed in each well and sealed with a 4 inch PVC cap. E-PERMS were chosen because they are not affected by the high humidity of the soil and can be read on site. Eleven E-PERMS were exposed for 24 hours and 31 were exposed for 48 hours. Radon levels ranged from 0 pCi/l (pico Curies per liter) to 873 pCi/l. The mean was 138 pCi/l and the average was 210 pCi/l. The

data indicate that there is a strong radon source in the foundation fill. Therefore the potential exists for elevated radon levels in the finished building. Radon was not a known health threat when the other buildings on campus were constructed so pre construction radon levels are not known. There is little data available from anywhere from which to draw comparisons between soil gas radon and building radon levels. Such comparisons are made very difficult because each site and each building are unique. However based on the results of previous testing on campus and our experience with over 800 radon tests in this area of West Virginia we would expect to see radon levels in the finished building in the 1-10% range of the soil gas measurements. It should be noted that the highest measured radon level in any building on campus is 9.6 pCi/l. This is about 1% of the highest soil gas radon level found at the new building site.

JOHN H. HULL, Dept. of Psychology, Bethany College
Bethany, West Virginia 26032. Final exams: Who
needs them?

Pearson product-moment correlation coefficients were computed across the past four years between students' scores on comprehensive final exams and their unweighted total scores on hour tests for introductory psychology (typical class size, 110 students) and statistics (typical class size, 30 students) courses. Correlation coefficients for the four years ranged from .63 to .90 for the statistics course, and from .75 to .84 for the introductory psychology course; all correlations were statistically significant ($p < .001$). Within the context of these courses, then, comprehensive final exams appear to add little additional information for determining final course grades, although other valid reasons for giving comprehensive final exams exist, and will be discussed.

Roger Neustadter
Concord College
The Decline of the Idea of Progress
In American Public Opinion

There seems to be a reaction against industrialism and the idea of history as progress at its most pervasive and compelling operation, that of ideology. However, the evidence for this probably does not convince the hardheaded social scientist who wants their data in precise numbers and proportions. As it happens there is a certain amount of evidence of the conventional kind for the view that the idea of history as progress has weakened its hold on populations in industrial societies.

Survey data over the last two decades indicates that citizens in the mid twentieth century have taken note of the baneful effects of unrestrained technological

growth--the pollution of the atmosphere, the spoilation and exhaustion of natural resources, and the depersonalization of the workplace. The essential element in the idea of progress--the belief in improved lives for oneself, one's children, and one's children's children has been undermined. Faith in progress has plummeted from earlier heights. The paper examines polling data on progress accumulated over the last two decades.

It concludes that for a growing number of people the present is no longer seen as the glorious culmination of a progression from the primitive to the refined and the enlightened. The idea of history as progress for many it would seem is losing its charm. The immanence of change for the better in man's estate no longer seems imminent. The future no longer winks seductively.

Mathematics

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PROBABILITY DISTRIBUTIONS AND THE DEFINITE INTEGRAL

Beginning with the most basic and elementary notions in probability, a mathematical model is developed for predicting the outcome of experiments based on assumptions about certain associated events occurring. This model is stated in the form of a continuous function and its accompanying graph. Then using this function and its graph, it is shown how the area under the graph can be used to calculate probabilities for various events.

HERBERT H. SNYDER, Dept. of Mathematics, West Virginia Institute of Technology, Montgomery, WV 25136. A nonlinear differential equation occurring in electron beam dynamics.

The equation in question arises in the theory of cylindrical electron beams focused by a static magnetic field which is periodic in the direction along the beam axis. In normalized and/or dimensionless variables, the equation (as found in several places in the literature) is $y'' + \alpha(1 + \cos 2x)y = 2\alpha Ky^{-3} + \beta y^{-1}$, in which α , β and K are non-negative real constants taking values (mostly) in the unit interval. However, the author's closer study of its derivation shows that the last term on the right should not be singular at $y = 0$, and a suitable correction is made. It is then shown that no regular solution can vanish if $K \neq 0$. Some boundedness properties will also be given.

HERBERT H. SNYDER, Dept. of Mathematics, West Virginia Institute of Technology, Montgomery, WV 25136. Some new non-resident graduate degree possibilities in the United States.

In spite of long-standing recommendations of such bodies as the Carnegie Commission on Higher Education (in, e.g., Less Time, More Options, ca. 1970), few American "old line" universities are willing to admit that a mature and well-motivated adult is capable of learning outside their walls. There are a few

exceptions, however, and also some new institutions of good
repute. In addition, there are many older institutions (of
unquestionable integrity) abroad, and available to American
students at reasonable cost. The author will enumerate several
such institutions by name, and intends to have some of their
literature available for distribution.

Geology

E. RAY GARTON, Mammoth Geo, Inc, PO Box 200, Barrackville, WV 26559 and FREDERICK GRADY, 1201 South Scott Street, Apt. 123, Arlington, VA 22204. CASS CAVE MASTODON Mammut americanum SETS RANGE AND ELEVATION RECORDS FOR WEST VIRGINIA.

The recent find of a well preserved juvenile mastodon tooth Mammut americanum in Cass Cave, Pocahontas County, West Virginia marks the 17th mastodon discovery for West Virginia. Including 9 mammoth discoveries, there have been a total of 26 "elephants" found in the State. Eight mastodons but no mammoths have been found in West Virginia caves. Other mastodon finds include Organ, Rapps and McFerrin Caves in Greenbrier County, 2 from Scott Hollow Cave in Monroe County, Bowden Cave, Randolph County and Big Springs Cave, Tucker County. This discovery is made even more significant because it extends the range of the mastodon into Pocahontas County. In addition this discovery extends the elevation range for mastodon in West Virginia to 2975 feet. Mastodons and Mammoths became extinct at the end of the Pleistocene thus the discovery is assigned a minimum age of 10,000 years.

EBERHARD WERNER, P. O. Box 795, Morgantown, WV 26507.
Geochemistry of a carbonate-depositing stream and spring in Mineral County, West Virginia.

A small stream, an unnamed tributary to New Creek in Mineral County, issues from a small spring on the west slope of New Creek Mountain, and has contributed calcium carbonate precipitate to travertine-marl deposits in its bed. New Creek Mountain is underlain by steeply dipping rock of the western limb of the Wills Mountain anticline, and bedding dip exceeds topographic slope. The spring results from groundwater overflowing an underground dam formed by a low-permeability bed within the upper part of the Devonian Helderberg Group of rocks. The stream flows for approximately 700 feet while descending about 200 feet. Over this length, there are both gaining and losing reaches as water is diverted underground through relatively permeable beds of the Oriskany sandstone to be once again forced to the surface by less permeable layers, or by underflow in the alluvium. Samples were taken at five equally spaced locations along the small stream as well as at New Creek and analyzed for the major inorganic constituents. Waters are relatively normal karst waters, of a calcium bicarbonate composition with an additional moderate sulfate concentration. All samples, especially those from the spring run, were found to be highly supersaturated with respect to calcite, including the samples taken at the spring orifice. Even so, loss of calcium carbonate through precipitation does not begin until the water has flowed 150 feet below the spring to an area of increased stream turbulence, and then continues to the junction with New Creek, at degrees of supersaturation in excess of ten times saturation with respect to calcite. Water analysis data and analyses of travertine and marl from the stream bed indicate that the material precipitating is probably a low-magnesian calcite. In the 600-foot length of stream over which precipitation occurs, the stream loses ten to fifteen percent of its dissolved calcium and bicarbonate, but little or none of the other ions present.

Zoology

DOUGLAS B. CHAMBERS, MICHAEL LITTLE and CHARLES E. PUCKETT, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755. Analysis of the Chromosomal Structure of the Longnose Dace, *Rhinichthys cataractae*.

The analysis of chromosome structure can often be used to determine hereditary characters unique to a species. The longnose dace (*Rhinichthys cataractae*) has long been known to hybridize with many other species, in many genera. The analysis of its chromosomal structure can provide useful information in determining the parentage of suspected *R. cataractae* hybrids. Karyotypes were prepared by allowing *R. cataractae* to swim in a 0.10% colchicine solution for 10-12 hours. The fish were then sacrificed and a suspension of gill epithelium was used to prepare slides. The slides were stained with giemsa and for c and r banding and for the Nucleolar Organizer Region. Photomicrographs were used to prepare ideograms and chromosome maps to illustrate unique cytogenetic features of the species. Initial analysis has determined the nucleolus organizer region of *R. cataractae*.

TIM HAYES and DONALD TARTER. Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Preliminary observations on the fall season food habits of a disjunct population of the central mudminnow, *Umbra limi* (Kirtland), in the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

The central mudminnow, *Umbra limi*, is mentioned on the Vertebrate Species of Concern List in West Virginia. The only population of the central mudminnow in West Virginia is found in the wetland habitat (ca 57 ha) of the Green Bottom Wildlife Management Area. This disjunct population is located 100 km from the nearest population in Hocking County, Ohio, and provides a unique opportunity for a food habit study outside its normal range. Mudminnows ($\bar{X}=25/\text{month}$) were collected from September to November 1991 by seining the vegetated, littoral zones of the wetland habitat. In the laboratory, quality and quantity of the monthly stomach contents were determined using the following calculations: (1) percentage frequency of occurrence, (2) average of volume percentages, and (3) percentage of total volume by weight. Comparisons will be made with other food habit studies of mudminnows in the literature.

MICHAEL LITTLE and DOUGLAS CHAMBERS, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Distribution of the Cheat Minnow, *Rhinichthys bowersi*, in relation to environmental quality.

The Cheat minnow, "*Rhinichthys bowersi*", is either a valid species or a river chub (*Nocomis micropogon*) X longnose dace (*Rhinichthys cataractae*) hybrid. With the exception of few records in the Great Lakes drainage, *R. bowersi* has been collected primarily in the Cheat River, its tributaries, and in adjacent streams of the Monongahela River system. Streams in these drainages are exposed to high levels of acid deposition and/or acid mine drainage. In this study we examined seven headwater streams of the Cheat River for the presence of *R. bowersi* and surveyed the fish communities associated with *R. bowersi*. Additionally, pH, acidity, alkalinity, and calcium hardness were measured from five Cheat River tributaries which are inhabited by *R. bowersi*.

KATIE MCGINN, TIM HAYES, TOM JONES, JOHN WIRTS, DONALD TARTER. Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Ichthyofauna of the Green Bottom Wildlife Management Area, Cabell County, West Virginia, with preliminary observations on the spawning activities of the bowfin, *Amia calva* Linnaeus.

The Green Bottom Wildlife Management Area is located along the Ohio River approximately 26 km northeast of Huntington, West Virginia. The area (ca. 338 ha) contains a valuable wetland habitat (ca. 57 ha) in southeastern West Virginia. A limited ichthyofauna exists in the wetland habitat due to fluctuating temperatures, water levels, and dissolved oxygen levels. The following fishes have been collected from the area: bowfin, central mudminnow, grass pickerel, carp, black and yellow bullheads, largemouth bass, bluegill, green sunfish, and black and white crappies. The only reproducing population of bowfin in West Virginia occurs in the wetland habitat. Preliminary observations on its spawning activities will be presented and compared with literature reports.

NANCY POWERS, ERICH EMERY, TIM HAYES, TOM JONES, DONALD TARTER. Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Low pH tolerance, under static bioassay conditions, of the crayfishes *Fallicambarus fodiens* (Cottle) *Cambarus diogenes* Girard from the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

The burrowing crayfishes *Fallicambarus fodiens* and *Cambarus diogenes* were experimentally tested under static bioassay conditions to

determine their tolerance to low pH. Crayfishes were exposed to four pH values (6.5, 5.0, 3.5, 2.0) in the laboratory investigation. The straight-line graphical interpolation method was employed to determine the pH at which 50 percent of the crayfishes survived after 96 hours. Results from this study will be compared to other low pH tolerance studies of crayfishes.

CHARLES E PUCKETT, MICHAEL LITTLE and DOUGLAS B. CHAMBERS, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755. Analysis of the Chromosomal Structure of the River Chub, *Nocomis micropogon*.

The analysis of chromosome structure can often be used to determine unique hereditary characters of a species. The river chub (*Nocomis micropogon*) is a species that hybridizes readily, both within and beyond its own genus. The analysis of its chromosomal structure can provide useful information in determining the parentage of suspected *N. micropogon* hybrids. Karyotypes were prepared by allowing *N. micropogon* to swim in a 0.01% colchicine solution for 10-12 hours. The fish were then sacrificed and a suspension of gill epithelium was used to prepare slides. The slides were stained with giemsa and for c and r banding and for the Nucleolar Organizer Region. Photomicrographs were used to prepare ideograms and chromosome maps to illustrate unique cytogenetic features of the species. Initial analysis has determined the nucleolus organizer region of *N. micropogon*.

DONALD TARTER, Dept. of Biological Sciences, Marshall University and DIANE NELSON, Dept. of Biological Sciences, East Tennessee State University. First records of tardigrades (Phylum: Tardigrada) from mosses on Dolly Sods Wilderness Area in the Monongahela National Forest, Tucker County, West Virginia.

Seven species of tardigrades were collected from mosses on Dolly Sods Wilderness Area located in the Monongahela National Forest in north-eastern West Virginia. Dolly Sods is a tundralike area that sits atop a ridge of the Allegheny Mountains, which form the eastern section of the 4,000-foot Allegheny Plateau. One-hundred and fifty inches of snow may fall in the winter, forming drifts that do not completely melt until May. Scattered in poorly drained depressions of the high plateau are soggy areas with continuous mounds of sphagnum and haircap mosses. The following species of tardigrades, including the state record *Macrobiotus tonollii* Ramazzotti, were found from mosses on rocks and the ground: *Hypsibius convergens* (Urbanowicz), *Macrobiotus harmsworthi* Murray, *M. hufelandi* Schultze, *M. richtersi* Murray, *Milnesium tardigradum* Doyere, and *Ramazzottius oberhaeuseri* (Doyere).

Physical stress may negatively affect reproductive cyclicity in females. Acute stress such as footshock or surgery have been shown to reduce circulating levels of gonadotropins in several species. The objective of this experiment was to assess the effects of forced running on the fertility of female laboratory rats. Sixteen adult laboratory rats were randomly assigned to receive either running (R:n=8, 6 min/day for 17 days) or non-running (NR:n=8). Rats were forced to run (0.25-0.35m/sec) on a treadmill (R) or were placed on the treadmill with the motor running, but the belt stationary (NR) to control for random vibrations. Daily vaginal smears were collected to determine reproductive cyclicity. Rats were mated (day 1 of pregnancy) on the first available day after 17 days of running, then implantations sites were counted (day 7 of pregnancy). There were no differences in cyclicity between R and NR rats. There were, however, fewer implantation sites in the R rats (3.38 ± 0.63) than for NR (10.63 ± 1.22 ; Fig 1). It is concluded that the stress of running resulted in an inability to maintain normal numbers of pups through day 7 of gestation.

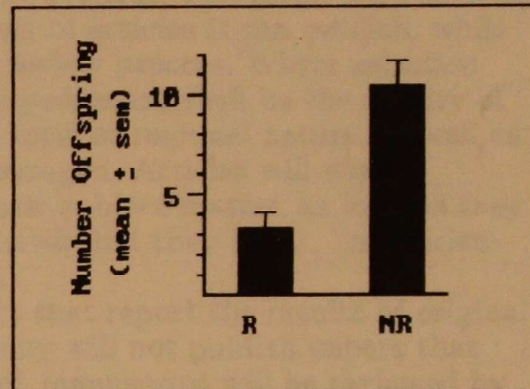


Figure 1 Number of implantations counted at necropsy in rats which ran (R) or did not run (NR).

JOHN WIRTS and DONALD TARTER. Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Food habits of the bowfin, Amia calva Linnaeus, in relation to age and seasonal changes in the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

The bowfin, Amia calva, is the only extant member of the Holostean family Amiidae. Only four isolated records of bowfin have been reported for West Virginia. These records are from flooded areas along the Ohio River or stocked ponds. Recently, we found the only reproducing population in West Virginia at the Green Bottom Wildlife Management Area (GWMA) in Cabell County. The GWMA ($38^{\circ}35'35''N$, $82^{\circ}14'55''W$) is located along the Ohio River 26 km northeast of Huntington, West Virginia. The area (ca. 338 ha) is a valuable wetland habitat (ca. 57 ha) in southeastern West Virginia. A proposed habitat modification to add marshland by building dykes has prompted this study. Information from the study will establish a baseline for food habits of the bowfin prior to habitat disturbance. Bowfin (7-17/season) were collected by hoop nets, traps, and seine.

In the laboratory, age classes were determined using the gular plate, and seasonal food habits were analyzed using the following calculations: (1) percentage frequency of occurrence, (2) average of volume percentages, and (3) percentage of total volume (by weight).

PROCEEDINGS OF THE WEST VIRGINIA
ACADEMY OF SCIENCE

INSTRUCTIONS TO AUTHORS
Revised February 1982

1. *General Policy*

The publications policy of the Academy is intended to implement the goal of publication of the *Proceedings* by the Academy, namely, stimulation of research on the part of West Virginia scientists and Academy members by providing an outlet for publication of their research results. Within the limits of available resources, the Academy will attempt to maximize the number of articles it can publish, while maintaining standards by the peer review process. Where selection must be made, the sole criterion for judgment shall be the quality of the research involved. Articles of a local or regional nature, as well as those of broader scope, will be encouraged. Articles will not be discriminated against because of their subject matter, as long as they satisfy the requirement of the By-Laws that they be "... of a scientific nature" (Section VII, Article 1).

The Academy will consider papers that report the results of original research or observation. The Academy will not publish papers that have been published elsewhere. Each manuscript will be reviewed by the Publications Committee and by referees. Manuscripts longer than 15 pages* of double spaced typed copy normally will not be accepted. Membership in the Academy is a requirement for publishing in the *Proceedings*. In the case of joint authorship, at least one author must be a member of the Academy, and the author presenting the paper must be a member of the Academy. No author, or co-author, may submit more than two papers for any volume of the *Proceedings*. Ordinarily, papers offered for publication must have been presented at the annual meeting of the Academy. Publication is not automatic. The *Proceedings* editors also solicit outstanding expository papers.

2. *Preliminary Abstract*

A preliminary abstract, summarizing the results of the investigation must accompany the application for a place on the program of the annual meeting. The preliminary abstract must be typed on a special form, available from the Academy officers or editor, and will be published in the first number of the volume for that year. There is a \$2 fee for each abstract submitted.

3. *Organization of Manuscripts*

Each manuscript shall start with an abstract (no more than 250 words) which should summarize the primary results. The following sequence is suggested for organizing a paper: Introduction, Materials

*The 15-page count refers to typewriter text and pages of figures, graphs, photos, and abstracts.

and Methods, Results, Discussion, Acknowledgments, and references cited. With the exception of the introduction, each division of the manuscript should be labelled. Sub-headings may be used. In general, the introductory abstract will replace a summary. This abstract should be suitable for sending to international abstracting services for immediate publication in the event that the paper is accepted for publication in the Proceedings.

4. *By-Line*

The author's name, department, institution, city, state, and zip code should follow the title.

5. *Form*

Manuscripts shall be typed double spaced on white bond paper. A dark undamaged ribbon should be used on typewriters in order to produce clear copy for the editor and the printer. Pages of copy should be numbered consecutively in the top right-hand corner of each page of the manuscript, preceded by the author's last name. *Two copies, the original typed copy and a Xerox*, together with a set of original photos, figures and/or drawings, should be given to the Section Chairman on the day of the Annual Meeting. Each table or figure should be supplied with a legend sufficiently complete to make the table or figure intelligible without reference to the text. Footnotes may be used in connection with tables and figures where necessary and may save space. Footnotes should be avoided wherever possible in the text itself. Complicated formulas should be prepared with care in a form suitable for *camera copy reproduction*. Avoid such formulas in a line of text.

6. *Illustrations and Special Symbols*

Line drawings should be carefully made on good rag paper for direct photo reproduction. Each figure should be numbered. While drawings may be of any convenient size, they will be reduced to 3 x 4 inches. Letters, symbols, and figures should be not less than 1 mm. high *after reduction* to printing size. In exceptional instances, a full page drawing (4½ x 6½ inches) may be used. Either original drawings or glossy photographs (mounted on illustration board with rubber cement) may be submitted. Photographic prints should be on glossy paper and have good contrast. Each drawing should be labelled on the back with the author's name and the appropriate legends. Camera copy will be used to reproduce mathematical formulas as far as practicable.

7. *Literature Cited*

References shall be collected at the end of the manuscript as "Literature Cited," and must be cited in the text.

The title of the papers cited and the inclusive page numbers must be given. References in the text may be either by year or by number. Examples: Hall and Campbell (1957) or [5]. Square brackets are recommended for references so that numbers in parentheses may be used to denote formulas in the text.

Example of a journal citation at the end of paper:

5. Hall, J. L., and R. Campbell. 1957. Polarization of ethanol in benzene. Proc. W.Va. Acad. Sci. 29:53-57.

Example of a book citation:

6. Stacey, M., and S. A. Barker. 1960. Polysaccharides of microorganisms. Oxford Univ. Press. London. 228 pp.

8. *Proof*

Galley proofs will be sent to authors for corrections. Make corrections on the margins of the proof. Proofreader's marks may be found in dictionaries, or in style manuals (e.g., "Style Manual for Biological Journals"). Changes in text after the manuscript is in galley proof are quite expensive and in general are not permitted. Galley proofs must be corrected and returned promptly (within ten days).

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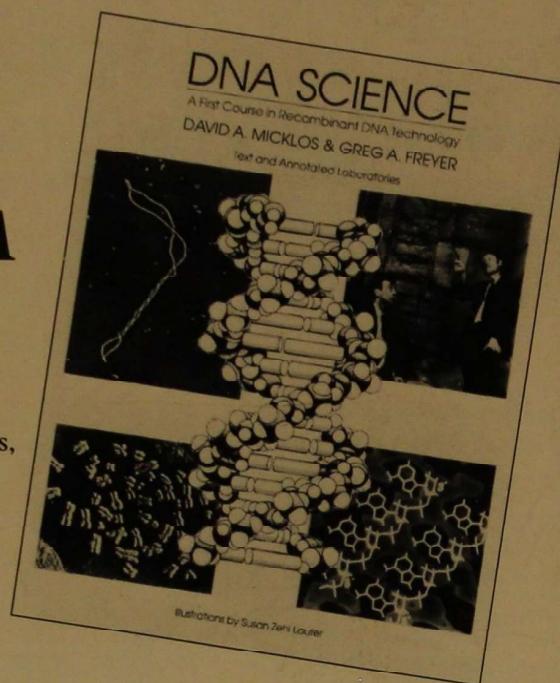
DNA Science: A First Course in Recombinant DNA Technology

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Columbia University

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