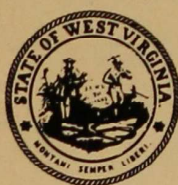


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**Abstracts of papers for
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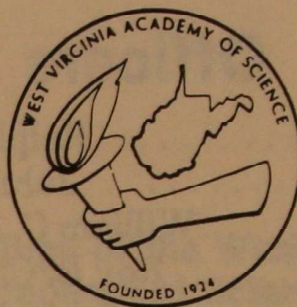
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Abstracts of Papers

for the 1987 Meeting

Biochemistry/Physiology

SANDRA MYA, RON E. GAIN, BRYAN LARSEN, Department of Microbiology, Marshall University School of Medicine, Huntington, West Virginia 25701.
Development of a High Performance Liquid Chromatographic Assay for Measuring Mezlocillin (Mez) in Serum or Tissue.

New derivatives of penicillin, including Mez, are finding increasing use in clinical medicine because of their broad spectrum of antibacterial activity. Sensitive assay methods are needed as tools for understanding the pharmacodynamics of these drugs. Serum and uterine cervix samples were available from 14 women who underwent hysterectomy and a chromatographic method was developed to assay these samples for Mez. Mez reference standard was diluted in water and chromatographed on a C18 reversed phase column using acetonitrile and phosphate buffer (1:3) as an eluent with uv detection at 210 nm. Mez furnished a discrete peak with a retention time of 2.4 minutes. The sensitivity of the assay was 0.1 $\mu\text{g/ml}$, and gave a linear response up to 100 $\mu\text{g/ml}$. The correlation coefficient for the standard curve was 0.9997. When reference standard was diluted in serum, the sensitivity of the assay was complicated by interfering compounds. We were able to remove these by acetonitrile precipitation followed by ether extraction. The sensitivity of the assay performed in serum was 3 $\mu\text{g/ml}$ and provided a linear response up to 100 $\mu\text{g/ml}$.

The patient samples were obtained from patients who had received 4 g of Mez intravenously. Serum levels ranged from 81.2 to 358 $\mu\text{g/ml}$ with an average of 207.5 $\mu\text{g/ml}$. When serum containing a known amount of Mez was homogenized for a length of time similar to that required to homogenize tissue, a detectable loss of Mez was observed and this was applied as a correction factor to the observed tissue levels. The average tissue level was 117.2 $\mu\text{g/ml}$ and ranged from 27 to 98% of the serum levels.

VERNON E. REICHENBECHER, JR., Dept. of Biochemistry, Marshall University School of Medicine, Huntington, WV 25504. Plasma protein variations in Spontaneously Hypertensive Rats.

The proteins present in the plasma of 12-16-week-old Spontaneously Hypertensive Rats (SHR) and their normotensive controls, Wistar-Kyoto Rats (WKY), have been analyzed by the extremely sensitive technique of two-dimensional polyacrylamide gel electrophoresis followed by color silver staining to visualize the protein spots. Several hundred proteins have been visualized by

this method. Inspection of the electropherograms has led to the discovery of two protein variants that appear to be associated with hypertension, and a third that appears to be a polymorphism associated with different breeding colonies of rats. SHR and WKY rats each contain alternate forms of a 37,000 dalton protein, with the form present in SHR exhibiting a more basic pI. SHR rats also exhibit a 32,000 dalton protein not found in WKY rats. In addition, SHR rats obtained from Charles River Laboratories and Harlan Sprague-Dawley, but not from Taconic Farms, contain a 31,000 dalton protein which is missing in WKY rats from all sources. These protein variations may provide clues to the causes and/or effects of hypertension and may be valuable as genetic markers for proneness toward the development of hypertension. (Supported by the American Heart Association, West Virginia Affiliate.)

GARY O. RANKIN, Dept. of Pharmacology, Marshall University School of Medicine, Huntington, West Virginia 25704-2901. Effect of adrenal demedullectomy on guanethidine tolerance.

Guanethidine is a potent antihypertensive drug which can produce profound decreases in blood pressure and heart rate. Guanethidine therapy is usually reserved, because of potential bothersome side effects, for times when conventional therapy has proven to be ineffective. Previous studies from my laboratory have demonstrated that spontaneously hypertensive rats (SHR) as well as other hypertensive rat models respond quickly to the antihypertensive properties of guanethidine. However, some hypertensive rats quickly become tolerant to the blood pressure lowering effects of daily guanethidine therapy. Early work suggested that the renin-angiotensin-aldosterone system contributed to the development of tolerance to guanethidine. However, even in rats treated with the angiotensin converting enzyme inhibitor captopril, blood pressure increased during 14 day guanethidine treatment. Therefore, other physiological systems appeared to be contributing to guanethidine tolerance. The purpose of this study was to determine the role of catecholamine release from the adrenal glands in the development of tolerance to guanethidine. Groups of male SHR (10 weeks old) were anesthetized (sodium pentobarbital, 40 mg/kg, intraperitoneally) and the adrenal glands exposed via flank incisions. In the sham-operated groups (SHAM; N=10), adrenals were exposed but replaced untouched. In the adrenal demedullectomy group (SHAD; N=7), adrenal glands were demedullectomized by pricking the capsule and squeezing the medulla from the gland. Animals were allowed to recover from surgery for 6 weeks after which systolic blood pressure and heart rate were determined twice weekly using tail artery

occlusion techniques. At 19 weeks of age, rats received guanethidine (50 mg/kg/day, intraperitoneally) daily for two weeks. Blood pressure and heart rate were determined on alternate days at least 20 hr after the last guanethidine dose. In SHAM rats blood pressure was significantly decreased by guanethidine on days 1 (-33 mm Hg) and 3 (-35 mm Hg), but was not significantly reduced by day 5. Heart rate was significantly reduced on all treatment days. In SHAD rats blood pressure remained lowered on treatment day 5 (-32 mm Hg) and was significantly lower than SHAM rats on treatment days 13 and 14. Although heart rate was significantly lowered on all treatment days, heart rate was decreased less in SHAD rats than SHAM rats after treatment day 5. These results suggest that release of catecholamines from adrenal glands plays an important role in the development of tolerance to guanethidine. The ability of adrenal demedullectomy to suppress late rather than early development of tolerance also suggests that adrenal catecholamines are more responsible for maintaining tolerance than initiating tolerance development to guanethidine. (Supported by a grant from the West Virginia Affiliate of the American Heart Association).

H.H. LO and G.O. RANKIN, Department of Pharmacology, Marshall University School of Medicine, Huntington, WV 25704-2901. In vitro nephrotoxicity of trichloroanilines.

Previous studies from our laboratory have demonstrated that monochlorinated aniline (CA) and dichlorinated aniline (DCA) derivatives are nephrotoxic to male Fischer 344 rats. The purpose of this study was to examine the in vitro nephrotoxic potential of four trichlorinated aniline (TCA) isomers, 2,3,4-, 2,4,5-, 2,4,6- and 3,4,5-TCA. Trichloroanilines were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI) and recrystallized from ethanol. Male Fischer 344 rats (200-250 g) were killed by cervical dislocation, kidneys were rapidly removed and renal cortical slices were prepared freehand. Approximately 100 mg of tissue were incubated with shaking in Krebs-Ringer phosphate buffer (pH 7.4) containing 5.0 mM potassium and 1.0 mM calcium at 25°C under a 100 % oxygen atmosphere in a Dubnoff metabolic shaker. Tissue slices were incubated for 30 min with vehicle, or an aniline prior to [14 C]-para-aminohippurate (PAH) or [14 C]-tetraethylammonium (TEA) addition and for 90 min with PAH and TEA. Aniline solutions were prepared from the purified anilines in ethanol such that the addition of 30 μ l of the aniline solution would yield the desired final concentration in the 3 ml of incubation media. Control samples had 30 μ l of ethanol only added to the incubation media. Accumulation of PAH and TEA by renal cortical slices was expressed as the slice to medium (S/M) ratio, where S equalled radioactivity (dpm)/g of tissue and M equalled the

radioactivity (dpm)/ml of incubation media. The results of this study demonstrated that all TCA decreased TEA uptake at a concentration of 10^{-4} M. All TCA except 2,4,6-TCA decreased lactate-stimulated PAH uptake at a concentration of 10^{-4} M. TEA uptake was decreased with 2,3,4- and 3,4,5-TCA at a concentration of 10^{-5} M. None of the four TCA isomers decreased basal PAH uptake; nevertheless, 3,4,5- and 2,4,6-TCA stimulated basal PAH uptake at concentrations of 10^{-5} M or greater. These results indicate that TCA are more nephrotoxic than CA or DCA in vitro. The order of the in vitro nephrotoxic potential for TCA was found to be 2,3,4-; 3,4,5- > 2,4,5- > 2,4,6-TCA. These results also suggest that the site of nephrotoxic action of TCA seems to be toward the later section, S₃, of the proximal tubule, since TEA accumulation was altered at lower TCA concentrations than PAH accumulation.

JOSEPH M. DADGARI and E. BOWIE KAHLE, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25701. Adaptive responses of enzymes of carbohydrate and lipid metabolism to exercise-training in the genetically obese LA/N-CP rat.

The ameliorative effects of exercise-training (EX) compared with sedentary conditions (SED) were studied in 45 LA/N-corpulent rats (20 obese and 25 lean phenotypes). Exercised groups were treadmill-trained for 60 min/day, 5 days/week and 13.5 meters/min over 10.7 weeks. Citrate synthase (CS) and 2-oxoglutarate dehydrogenase (OGD) activities were determined in red vastus and soleus muscles and in liver as monitors for exercise-related changes in oxidative capacity. Glucose-6-phosphate dehydrogenase (G-6-PD) and malic enzyme (ME) activities in liver were determined to monitor the level of lipogenesis. Obese animals (EX and SED) when compared to lean (EX and SED) exhibited higher levels of CS ($P < 0.05$) in both muscles, and higher levels of OGD, G-6-PD and ME ($P < 0.05$) in liver. Exercise-training increased the activities of OGD and CS in the red vastus muscle of obese EX vs. SED ($P < 0.05$). Although not significant at $P < 0.05$ level, activities of OGD and CS in the soleus also tended to be higher in obese EX vs. SED groups. Exercise-trained obese animals (EX vs. SED) exhibited reduced activities of G-6-PD and ME ($P < 0.05$) in the liver, indicating reduced lipogenesis. No indications of training effect on lean (EX vs. SED) groups were recorded for the enzymes tested. Thus, an exercise regimen sufficient to train this strain of obese rat may not produce a training effect in its lean phenotype. Beneficial effects of exercise-training on the obese condition are suggested by: 1. increased activities of oxidative enzymes leading to greater thermogenesis; and 2. reduced activities of lipogenic enzymes indicating reduced lipid storage.

WEBB, DOSHIA, BRUCE RIPLEY, H. WAYNE ELMORE and
ALAN WHITE. Dept. of Biology, Marshall
University, Huntington, West Virginia 25701.
Optimized growth and regeneration of gametophytes
in sporophytic Bracken Fern tissue cultures

Tissue cultures were established by transferring roots of axenically cultured sporophytes of the Bracken Fern, Pteridium aquilinum (L.) Kuhn var. Latiusculum to Knudson's medium containing 2% sucrose, 2.0 ppm 2,4-D and 0.2 ppm kinetin in darkness. Approximately 100 isolates of callus were subcultured. Most of the callus lines were slow-growing and dark brown in color. One rapidly-growing clone was selected for study. A series of experiments was completed to determine effects of antioxidants including polyvinylpyrrolidone, L-ascorbic acid, L-cysteine HCl, glutathione and sodium selenite on growth and browning. All of the antioxidants stimulate growth with the exception of glutathione which was inhibitory. The results suggest that phenolic production and possibly other oxidative processes reduce growth. The callus is now routinely subcultured on medium containing 5.0 ppm ascorbic acid. Experiments were conducted on the regeneration of root callus. Pieces of callus were transferred to medium containing 0-4% sucrose without 2,4-D, kinetin or ascorbic acid. Gametophytes regenerated in large numbers from callus grown on 0% sucrose. Concentrations above 1.0% resulted only in extensive dark brown callus growth. The callus consisted of undifferentiated spherical-elliptical cells parenchyma cells. Sucrose exerts a marked influence on the developmental pattern and is the key factor in controlling the regeneration of gametophytes from sporophytic root callus.

RIPLEY, BRUCE, DOSHIA WEBB, H. WAYNE ELMORE
and ALAN R. WHITE. Dept. of Biology, Marshall
University, Huntington, West Virginia 25701.
Nutritional and hormonal requirements for
Bracken Fern cell suspension culture

A rapidly-growing callus culture was established from roots of the Bracken Fern, Pteridium aquilinum, on solid Knudson's medium containing 2% sucrose, 2 ppm 2,4-D, 0.2 ppm kinetin, 5. ppm ascorbic acid and 1.2% agar. Liquid medium of the same composition lacking agar was used in an effort to establish a cell suspension culture without success. Growth was dramatically increased on Knudson's medium supplemented with 10% coconut milk. Attempts to develop a defined medium without coconut milk have centered around studies on the effects of combinations of mixtures of six vitamins, six cytokinins and casein hydrolysate. Casein hydrolysate is the most important factor. The culture is now maintained at 23°C in darkness on a shaker at 175 rpm in 0.5 or 2.0 l Wheaton shake flasks containing

Knudson's medium supplemented with 2% sucrose, 5 ppm ascorbic acid, 2 ppm 2,4-D, 100 ppm inositol and 10 ppm casein hydrolysate. Cultures are subcultured at 14-day intervals. Growth increases several hundred percent over a 2-3 week experimental period. As far as authors are aware this is the first report of a sustainable fern cell suspension culture.

MARCIA A. HARRISON. Dept. of Biology, Marshall University, Huntington, West Virginia 25701
Physiology of the wounding response in plants.

Chemical and physical stresses increase ethylene production in most plant tissues. This stimulation of ethylene production is part of the wounding response in plants and may be signalled by a 'wounding factor'. In 7-day-old etiolated stem segments of pea, a transitory increase in wound ethylene occurs after a lag of 26 min and reaches a maximum 56 min after excision (Saltveit and Dilley, Plant Physiol 61:447-550, 1978). It was found that this wound ethylene response can be reduced from 50 to 25 $\mu\text{l g}^{-1} \text{min}^{-1}$ by excising etiolated pea stem segments under water and washing them in an aerated water bath for 2 min. The stem segments sandwiched between moist filter paper in small vials can be assayed for ethylene production 50 to 65 min after excision. Extracellular material centrifuged from stressed (heated) pea stem segments can be applied to the filter paper circles and analyzed for their activity in stimulating the wound ethylene response in this pea stem segment bioassay. Extracellular centrifugate from segments heated in a 42°C water bath for 5 min stimulated ethylene production in the bioassay compared to control segments kept in a 23°C water bath. It is proposed here that a 'wounding factor' is released from the cell wall or into the cell wall after heat stress and signals the production of wound ethylene in plants.

Biology

DANA ANDENS and E.C. KELLER, JR. Department of Biology, West Virginia University, Morgantown, WV 26506. County Cardiovascular Mortality Trends, by Sex, in West Virginia --- 1959 through 1982.

Cardiovascular mortality trends were determined, by sex, for each county of West Virginia. These trends included all types of cardiovascular mortality and also, the four major sub-groupings of cardiovascular mortality viz., cerebrovascular, heart disease, hypertension, and arteriosclerosis. The data were obtained from the mortality records of the WV Department of Health for the years 1959 through 1982. The data were expressed as mortality per 1000 as adjusted by the population estimates of the respective county.

In general, for major cardiovascular mortality, 42% of the counties showed decreasing trends in males while 16% of the counties showed increasing trends for males. In contrast, again for major cardiovascular mortality, only 11% of the counties showed decreasing trends in females, while 46% of the counties showed increasing trends for females.

For cerebrovascular mortality, 33% and 55% of the counties showed decreasing trends for male and female mortality, respectively. In contrast, for heart disease mortality 55% and 33% of the counties showed increasing trends for male and female mortality, respectively.

There were essentially no trends for hypertension mortality and arteriosclerosis mortality in the 55 counties.

BEVERLY FOOSE and E.C. KELLER, JR. Department of Biology, West Virginia University, Morgantown, West Virginia 26506. County Trends, by Sex, for Major Cancer Mortalities in West Virginia --- 1959 through 1982.

Trends were determined for general cancer mortality and three major cancer sub-groupings viz., respiratory, digestive/peritoneum, and genitourinary cancers, by sex, for each West Virginia county. The data were obtained from the mortality records of the WV Department of Health. The data were expressed as mortality rates per 1000 as adjusted by the respective county population estimates for those years. All three groups of cancers were categorized as having increasing, decreasing, or no trends for each of the counties.

In general, there were few trends for female digestive/peritoneum cancers. There were only two counties with increasing trends and two with decreasing trends. The same generalization held for genitourinary cancers, with two counties having increasing trends

and one with a decreasing trend. In regard to respiratory cancers in females, 38% of the counties were found to have increasing trends. The main clustering of those counties with increasing trends occurred in the southern coal mining region. No decreasing trends were observed for female respiratory cancer.

Generally, no trends were found for digestive/peritoneum cancers, in males. However, one county had an increasing trend and four had decreasing trends. Regarding genitourinary cancers in males, 15% of the counties showed increasing trends and one county showed a decreasing trend. Most of the counties with increasing trends of genitourinary cancer in males were found along the eastern banks of the Ohio River. Respiratory cancer mortality in males showed increasing trends in 78% of the counties. Generally, the counties showing no trends in male respiratory cancer mortality were those along the eastern border of the State. No decreasing trends were observed for male respiratory cancer.

A.R. MOORE and E.C. KELLER, JR. Department of
Biology, West Virginia University, Morgantown, WV
26506. Generalized Environmental and Socio/
Economic Models for Major Mortality Groupings in
West Virginia.

There are four major mortality groupings in West Virginia viz., cancer mortality, infant mortality, pulmonary-accident mortality, and respiratory-homicide mortality. In this study, three models were constructed for the four mortality groupings. Major environmental, socio/economic, and medical indices constructed from the data of the counties of West Virginia were used as the independent aspects of the model. The mortality data were obtained from the West Virginia Department of Health. The environmental, socio/economic, and medical data were obtained from the U.S. Environmental Protection Agency, WV Department of Commerce, WV Geological and Economic Survey, and certain projects done at West Virginia University.

Factor analysis was performed on some 40 mortality classes chosen for this study. The four groupings determined by factor analysis were: 1) malignant neoplasms, cancer of the digestive tract, cancer of the respiratory tract, and cancer of the genitourinary tract, referred to as "cancer mortality", 2) mortality in early infancy, death under 1 year of age, death under 28 days of age, mortality due to birth injury, difficult labor, and premature birth, collectively categorized as "infant mortality", 3) homicide, death due to bronchitis, emphysema, and asthma, and mortality by pneumoconiosis due to silica and silicates, referred to as "homicide and respiratory mortality", and 4) deaths due to accidents, the combined effects of influenza and pneumonia, and death due to pneumonia only, referred to as "pulmonary and accident mortality".

Stepwise regression was used to determine possible associations between environmental, socio/economic, and medical indices constructed from factor analysis of the non-mortality data bases and the four

major mortality indices constructed by the use of factor analysis as given above.

For the cancer mortality index, the number of children attending public schools per 1000 and the number of illegitimate births per 1000, accounted for approximately 25 percent of the variation in the model. The relative number of prenatal visits and illegitimate births accounted for nearly 37 percent of the variation in the infant mortality grouping. The homicide and respiratory mortality group was found to be associated with the relative number of prenatal visits, the percentage of surgical services used in the counties, and the number of illegitimate births. These three variables accounted for 50 percent of the variability in the respiratory and homicide mortality index. The pulmonary and accident mortality grouping was found to be associated with the percentage of patients in hospitals over the age of 65 years and the relative number of doctors in the counties. These two variables accounted for approximately 26 percent of the variation in the pulmonary and accident index.

TAMMY TROITINO and E.C. KELLER, JR. Department of Biology, West Virginia University, Morgantown, WV 26506. Associations Among Genetic, Environmental, Socio/Economic, Mortality and Disability Aspects of the Counties of West Virginia.

Gene frequencies of ten single gene human traits were estimated, by county, for nine dominant/recessive traits, viz., freckles, pigmented iris, bent little finger, Darwin's ear, widow's peak, long palmar muscle, tongue rolling, and mid-digital hair; and a co-dominant trait of hair type (curley, straight, or wavey).

The county gene frequencies were analyzed with a set of environmental, socio/economic, human mortality, and disability indices (which were) created by factor analyses using data from the WV Department of Health, the WV Geological and Economic Survey, US EPA, WV Department of Commerce, and certain environmental projects of West Virginia University. Stepwise regression analyses were used to assess possible relationships among the ten gene frequencies and factor indices describing major characteristics of the State. The genetic data were gathered by students at West Virginia University and Marshall University.

Five environmental indices viz., particulates, natural water alkalinity, drinking water hardness, and municipal water usage, showed varying degrees of association with five of the genetic variables. Two health related indices were also associated with five gene frequencies viz., major medical care and the rates of illegitimate births/prenatal care visits.

In an assessment of the gene frequencies of the ten traits with other human related indices it was found that eight of the gene frequency estimates were found to be associated with respiratory mortality and death due to various types of impairments.

L. Morgan,* (S.R. Lasky), C.T. Roberts, W.L. Lowe, D. LeRoith. West Virginia State College, Institute, WV 25112, and NIH/NIDDK/DB, Bethesda, MD. 20892. SEQUENCE HETEROGENEITY AT THE 5' END OF RAT INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) cDNA'S. DNA hybridization analysis of rat liver cDNA clones has revealed heterogeneity at the 5' ends. We have confirmed their expression in hepatic and extra-hepatic tissues with solution hybridization/RNase protection assays using an antisense Riboprobe which spans the divergent regions. We have further characterized these differences by cDNA sequence analysis. These experiments reveal that three 5' variants are differentially regulated with respect to tissue specificity and response to growth hormone. These data suggest that the expression of rat IGF-1 is controlled in a tissue specific fashion through hormonal control of mRNA processing.

W. T. Seaman*, (S.R. Lasky), C.T. Roberts, Jr., W.L. Lowe, Jr., D. LeRoith. West Virginia State College, Institute, WV 25112 and NIH/NIDDK/DB, Bethesda, MD. 20892. MOLECULAR CLONING OF RAT IGF-1 cDNA'S; DIFFERENTIAL mRNA PROCESSING AND REGULATION BY GROWTH HORMONE. Two classes of IGF-1 cDNA's were isolated from an adult rat liver library using a human IGF-1 cDNA probe. The two types of rat IGF-1 cDNA differed by the presence or absence of 52-bp insert which altered the derived C-terminal amino acid sequence of the E peptide, but not the 3' untranslated region or the sequence coding for the mature IGF-1 protein. Probes derived from these cDNA clones were used to elucidate the regulation of IGF-1 mRNA levels by growth hormone in various tissues of hypophysectomized rats.

C. Shultz*, (S.R. Lasky), L. Burgess, G. Roberts, C.T. Roberts. West Virginia State College, Institute, WV 25112 and NIH/NIDDK/DB, Bethesda, MD. 20892. MOLECULAR CLONING AND CHARACTERIZATION OF RAT GENOMIC DNA'S CODING FOR INSULIN-LIKE GROWTH FACTOR 1 (IGF-1). Three independently isolated genomic clones that contain the sequences coding for IGF-1 have been isolated from a Charon 4a phage library constructed with rat liver DNA partially digested by Eco RI. These clones contain fragments which hybridize to cDNA's from the 5' untranslated, the coding, and 3' untranslated regions and span greater than twenty kilobases of genomic DNA. We have constructed restriction maps of these DNA's and defined the intron/exon boundaries and a putative 5' flanking region that should contain sequences involved in the regulation of IGF-1 gene expression. This work is preparatory to the investigation of the regulation of IGF-1 expression by cis-acting and trans-acting factors in hepatic and extra-hepatic tissues.

Botany

RODNEY BARTGIS, Dept. of Natural Resources, P.O.
Box 67, Elkins, West Virginia 26241. Distribution
and Status of *Arabis serotina* Steele in West
Virginia.

Arabis serotina Steele (Brassicaceae) is a biennial endemic of Virginia and West Virginia shale barrens. It is currently under review for possible inclusion on the U. S. Threatened and Endangered Species List. A review of its status in West Virginia was recently completed. Although originally reported from nine West Virginia counties, most records were of misidentified *Arabis laevigata* var. *burkii*. *Arabis serotina* was found to be restricted in West Virginia to the Greenbrier Valley in Greenbrier County, and the South Fork Valley in Hardy and Pendleton Counties. Only twelve populations were found, with a total population of less than eight hundred individuals. Eighty percent of the individuals occur on only three barrens. The most severe threat appears to be predation by deer, which affected up to 70% of the individuals in some populations. Some protective measures have been taken but the species appears to warrant federal protection.

ROGER G. SEEBER, JR., JUDITH A. HORVATH, DAVID
F. BLAYDES
Biology Dept. West Virginia University
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Inhibition of Light Sensitive Lettuce Seed
Germination by Ethanol

Light sensitive lettuce (*Lactuca sativa*) seeds can be induced to germinate in darkness with the addition of cytokinins. Application of benzyladenine (BA), a synthetic cytokinin, to these seeds can increase germination to a point nearly equaling percentages found under light conditions. It was found however, that the presence of ethanol greatly retards this benzyladenine stimulated germination. Conditions at the time of sowing were as follows: 100 seeds sown on 2 sheets of filter paper already moistened with 4ml 10-4M BA, in darkness. The ethanol concentration in the BA solution was 0.75%. Less than 5% of the ethanol treated seeds germinated after 48 hours compared to 91% for the

nonethanolic cytokinin solution. Ethanol at this concentration seems to inhibit or delay the onset of seed germination in this system.

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

The following species of vascular plants have been recorded at the West Virginia University Herbarium as additions to the flora of West Virginia: Diplachne fascicularis (Lam.) Beauv., Sporobolus clandestinus (Biehler) Hitchc., Paspalum fluitans (Ell.) Kunth., Carex albolutescens Schwein., Betula populifolia Marsh., Salsola collina Pallas, Cerastium semidecandrum L., Nelumbo nucifera Gaertn., Astragalus neglectus (T. & G.) Sheld., Hypericum pyramidatum Ait., Ludwigia polycarpa Short & Peter, Acanthopanax sieboldianus Makino, Lonicera maackii (Rupr.) Maxim. and Solidago rigida L.

LOY R. PHILLIPPE, 203 Fisher Avenue, Carthage, Tennessee 37030 and JOSEPH ELY, Undergraduate Student, Natural Science Department, Salem College, Salem, West Virginia 26426. A Checklist of the Vascular Plants from the Murphy Preserve, Ritchie County, West Virginia.

An inventory of the vascular flora from the Murphy Preserve, Ritchie County, West Virginia resulted in a collection of 464 species encompassing 281 genera in 91 families. An annotated checklist of each species is given followed by a brief note concerning the habitat in which it was collected, the collector's initials, and collecting number. Following the collecting number in parenthesis are st (smaller tract) or lt (larger tract), the 117 hectare Murphy Preserve is divided by about 1.15 kilometer into a northern smaller tract (42.5 hectare) and a larger tract (69.2 hectare). The specimens are deposited in the herbarium of Eastern Illinois University, Charleston, Illinois. A brief history and description of the Murphy Preserve is also given.

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554 and MATTHEW T. GALL, Dept. of Forestry, West Virginia University, Morgantown, West Virginia 26506. The bark surface of living trees as a microhabitat for corticolous Myxomycetes.

The bark surface of living trees often supports a diverse array of living organisms, including a distinct group of "corticolous" Myxomycetes (plasmodial slime molds). Considerable variation exists among different species of trees as to their bark characteristics, including such things as pH, texture, nutrient concentrations, and water-holding capacity. Presumably, the differences that exist in the communities of corticolous Myxomycetes present on the bark surfaces of various species of trees are the result of this variation. In the present study, which was carried out in the Mountain Lake area of southwestern Virginia, the distribution patterns of corticolous Myxomycetes obtained in moist chamber culture from bark samples of 15 different species of trees were examined using the techniques of multivariate analysis. The results of this analysis suggest that microenvironmental complex-gradients of bark pH and bark texture are the major factors determining just what species of Myxomycetes are likely to be present on the bark surface of a particular tree. Of the tree species considered in this study, white oak (Quercus alba) and pignut hickory (Carya glabra) were found to support the most diverse communities of corticolous Myxomycetes, whereas red spruce (Picea rubens) was found to have the least diverse communities.

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554. Patterns of occurrence of Myxomycetes in the upland forests of southwestern Virginia.

A study of the distribution and occurrence of Myxomycetes (plasmodial slime molds) in the upland forests of the Mountain Lake area of southwestern Virginia was carried out during the period of 1982-86. Primary emphasis of the study was on analyzing patterns of microhabitat occupation, sporulation phenology, and species diversity of the Myxomycetes occurring in five different forest communities that occupied different relative positions (from subxeric to mesic) with respect to an environmental moisture complex-gradient. A high degree of similarity was found to exist among the communities for species composition of Myxomycetes. However, absolute abundance, species richness, and species diversity (as computed with Shannon's formula) were higher for the more mesic communities. In general, Myxomycetes appear to be rather opportunistic organisms, occupying those microhabitats suitable for their growth and development as these become available to them. Nevertheless, considerable resource partitioning

among species would seem to exist, since characteristic patterns of microhabitat occupation and sporulation phenology were noted for most quantitatively important species.

JOHN C. LANDOLT, Div. of Science and Mathematics,
Shepherd College, Shepherdstown, West Virginia
25443 and STEVEN L. STEPHENSON, Dept. of Biology,
Fairmont State College, Fairmont, West Virginia
26554. Cellular slime molds from West Virginia
caves.

A study of the occurrence and distribution of dictyostelid cellular slime molds (CSM) in two West Virginia caves was carried out during the period of 1985-87. Samples for CSM isolation were collected from Whittings Neck Cave in Berkeley County and Bowden Cave in Randolph County. Five different species (Dictyostelium aureo-stipes, D. minutum, D. mucoroides, D. sphaerocephalum, and Polysphondylium violaceum) were recovered, three of which occurred at both localities. In general, the distribution of CSM in caves appears to be rather "patchy," since they were isolated from only 56% of our samples. However, in the microsites where they do occur, these organisms exhibit surprisingly high levels of abundance and diversity and thus would seem to represent an important element in the microflora of West Virginia caves.

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont
State College, Fairmont, West Virginia 26554,
HAROLD S. ADAMS, Dabney S. Lancaster Community
College, Clifton Forge, Virginia 24422, and
MICHAEL L. LIPFORD, Dept. of Conservation,
Richmond, Virginia 23219. Persistence of chestnut
in the understory of upland forest communities of
western Virginia.

American chestnut (Castanea dentata), formerly a dominant or codominant tree in the upland forests of much of eastern North America, was almost completely eliminated as a canopy species by the chestnut blight during the first half of this century. However, chestnut has managed to survive in the understory by repeatedly sprouting from the root systems of blight-killed trees. In the present study, the distribution of chestnut as a component of the understory in upland forest communities was investigated in three different areas of western Virginia. Two of these areas (the Mountain Lake region of Giles County and eastern Alleghany County) are within the Ridge and Valley physiographic province and the third (Shenandoah National Park) is located in the northern Blue Ridge. Quantitative data on composition and structure of the vegetation and various topographic variables were analyzed using the techniques of multivariate analysis in an effort to determine the present distributional relationships of chestnut in the communities being studied. The results of this analysis indicate that the present

distribution of chestnut in post-blight forest communities is most strongly correlated with elevation and site moisture conditions. For example, the species is much less abundant (only 192 stems/ha) in forest communities in Alleghany County (mean elevation of 39 sampled stands = 645 m) than is the case for either Shenandoah National Park (532 stems/ha) or Mountain Lake (1008 stems/ha), where sampled stands occur at mean elevations of 822 m (20 stands) and 1079 m (43 stands), respectively. Maximum density of chestnut sprouts (more than 2500 stems/ha for some stands at Mountain Lake) was found to occur on subxeric sites, particularly those at mid- to upper slope positions on southern exposures. In all three areas, chestnut was conspicuously absent from most mesic sites.

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554 and HAROLD S. ADAMS, Dabney S. Lancaster Community College, Clifton Forge, Virginia 24422. A comparative ecological study of "healthy" and "declining" stands of red spruce in the Spruce Knob area of Pendleton County, West Virginia.

Quantitative data from a number of studies recently conducted in high-elevation spruce and spruce-fir forests at various localities throughout the eastern United States indicate that red spruce (*Picea rubens*) has shown a pattern of reduced growth and increased mortality since the 1960's. The exact cause of this decline is still unknown, although certain anthropogenic factors such as airborne pollutants may be involved. However, the possibility that various natural factors are responsible cannot be ruled out. In the present study, conducted during the 1986 field season in the Spruce Knob area of Pendleton County, two stands of red spruce--one apparently "healthy" and the other "declining"--were intensively studied in an effort to more completely quantify the extent and nature of the symptoms associated with spruce decline in West Virginia. Both stands occupy positions on the same ridgetop, occur at comparable elevations (1433 and 1463 m), and are (based on cored trees) approximately the same age. However, dead spruce trees represented 57.6% of all stems greater than 10 cm DBH and 54.6% of the total basal area in the declining stand, whereas the corresponding figures for the healthy stand were 12.9% and 8.0%, respectively. Dendroecological (tree-ring) analysis indicates that trees in both stands exhibited similar patterns of growth during the period of 1940-70, but since that time growth rates have increased in the healthy stand and markedly decreased in the declining stand.

MARK B. WATSON, ALAN R. WHITE, CHERYL E. WILLIAMS, JAMES P. GILL, H. WAYNE ELMORE, Department of Biological Sciences, Marshall University, Huntington, West Virginia 25701. Pteridium Polysaccharides.

The polysaccharides of fern cell walls have long been thought to be similar to higher plants. Callus cultures from Bracken fern, *Pteridium aquilinum* were transferred to a liquid medium and optimal conditions determined. (See paper Webb et al, this meeting) Vigorously growing cells were isolated, washed and treated with amylase. The polysaccharides were extracted with either alkali or Endopolygalacturonase (EPG). The alkali extractions included ammonium oxalate, a 0.5M - 4.0 M gradient of KOH and water. Each of the fractions were separated with DEAE Sephadex and the glycosyl composition analysed with GC using the alditol acetates procedure. Fern wall polysaccharides were compared to rhamnogalacturonan II (a typical higher plant pectin) which contains several characteristic sugars. (i.e. methyl-fucose, methyl-xylose, apiose, and aceric acid) The fern cell walls contained small amounts of the methylated sugars. Aceric acid and apiose were both present in significant amounts. A typical carbohydrate composition was: 72.9% glucose, 12.8% galactose, 6.1% arabinose, 3.8% xylose, 2.2% rhamnose, 1.1% fucose, 1.1% mannose. The low percentage of mannose is not consistent with the presumption (Preston, 1974, Phys. Biol. Plant Cell Walls, p.54) that glucomannans are the major hemicellulosic component of fern cell walls.

Chemistry/ Computer Science

Rita K. Eggleton and B. DasSarma

Department of Chemistry, West Virginia State
College, Institute, WV 25112. Evaluation of
Some Priority Pollutant in Indoor and Outdoor
Ambient Air.

Methods have been developed to measure some priority pollutants in ambient air. Constituents being analyzed include acetone, acrylonitrile, arsenic, benzene, beryllium, cadmium, chloroform, 1,2-dichloroethane, dichloromethane, ethyl benzene, lead, nickel, toluene, trichloroethylene and xylenes. Metals were evaluated by atomic absorption spectroscopy of concentrates in aqueous acids. Volatile organic compounds (VOC) were collected on active charcoal, and desorbed with benzyl alcohol and 1,2-dichlorobenzene. Capillary column gas chromatography of the extracts and headspace of the extracts were used for analysis of VOC.

Chituru Wokpara, Department of Agriculture,
Quality Control Division, State Capitol,
Charleston, WV 25305, and B. DasSarma, Department
of Chemistry, West Virginia State College,
Institute, WV 25112. An Alternate Method for
Preparing Standard Gas Mixtures.

Standard gas samples containing low level volatile organic compounds (VOC) and other air pollutants are required for routine calibration and analysis of ambient air. These are essential both for method development as well as for calibration of detectors in the development of gas chromatography. The three existing methods are (1) static method of adding known amounts into a storage vessel, where all the constituents are present as vapor, (2) dynamic method of delivering low-level component(s) by a continuous flow controller to measured volume of nitrogen or air, and (3) the more recent devices of controlled permeation of the minor component(s) in vapor form through specially constructed plastic or diffusion windows in sealed tubes. An alternate method involving partition coefficient between the vapor and the liquid phases in a solvent with low volatility will be compared with the other methods for preparation of low-level standard gas samples of chlorohydrocarbons in benzyl alcohol.

JAMES B. HICKMAN, Dept. of Chemistry, West Virginia University, Morgantown, WV 26506-6045.

The physical chemist as customer and creator of literature.

The author organized and taught for 25 years a course in use of the literature of science. He gives these 'commandments' whose rationale will be explained:

I. Senior person who'll direct long series of related projects should always list his own name first in author lists.

II. Senior person should 'police' the manner in which abstracting and review sources treat the work being published. If usually reported in mixed-up fashion, possible that senior not reporting clearly. (If one reference source only mixes up the work, may indicate reference source of low value).

III. As work progresses over years (don't wait too long) it may be that certain publications should be indexed under word(s) contained neither in original article nor in abstract (you invented world's best widget in article 3, but didn't know it -- 'widget' neither in article not in abstract -- if you act fast, you can get article indexed under 'widget' in addition to other listings.)

As a 'consumer' of literature, the 'senior' should practice him (her) self "working up" data before accepting them as entirely valid. Example: if programmable calculator like HP-67 or a simple PC is available, vapor pressure of liquids can be tested by noting consistency and smooth rate of change of ΔH^* from one pressure region to another. (Students can be 'broken in' to such practices by having them 'work up' both statistically and 'what real physical aberration is indicated?' masses of data from (e. g.) ACS Monographs like Properties Ord. Water Substance.) (* Calculated by integrated Clapeyron-Clausius equation)

A "big-shot proof" "speaker squelcher" developed by the author in 1968-69 will be demonstrated with the author as "squelchee."

JAMES J. SHIREY, JR., Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506. Methodology for soil pH analysis in a variety of soils from northern West Virginia.

Soil pH is difficult to accurately measure because it is dependent, in part, on the ionic strength of the sample. To examine this hypothesis, soil pH was measured using various types and concentrations of chloride-salt solutions and soil-solution ratios in four soils representing a wide range of initial ionic strengths (limestone, agricultural, forest, peat). The results indicated that pH values were: 1) 0.25 and 1.0 unit lower in 0.01 M and 1.0 M salt solutions, respectively, than in distilled water regardless of the soil type; 2) generally lower in divalent-salt solutions (Mg^{2+} , Ca^{2+}) than in monovalent-salt solutions (K^+); and 3) 0.7 unit lower

in a 1:1 soil-solution mixture than in a 1:10 soil-solution mixture, except for the water-saturated peat and disturbed agricultural soil. Ionic strength of the solution must be considered in soil pH analysis, even in soils with high initial ionic strength. Therefore, while soil pH is commonly measured in distilled water solutions, the most accurate procedure would employ a 1:1 soil-solution ratio using 0.01 M calcium chloride salt solution.

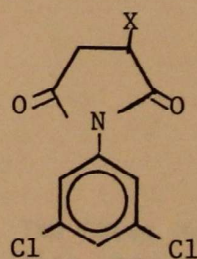
JOHN H. PENN, ELIZABETH D. COX, and AMRIT SINGH,
Department of Chemistry, West Virginia
University, Morgantown, WV 26506. Protonation of
Radical Anions as an Important Mechanistic
Pathway for Dechlorination of Chloroaromatics.

The lifetime of several chloroaromatic radical anions have been determined in acetonitrile as a function of the water concentration. The lifetime of the radical anions may be determined by monitoring the yield of photochemical product formation as a function of an added electron acceptor. This technique has been described previously (J.H. Penn and E.D. Cox, *Journal of Organic Chemistry*, 51, 4447 (1986)). The addition of water dramatically accelerates the rate of dechlorination in some cases. The rate of dechlorination of the radical anion may be expressed in the form $k_{dt} = k_{dt}^0 + k_w[H_2O]$ where k_w is approximately $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This is exceedingly close to the diffusion controlled rate. These results indicate that certain chloroaromatic radical anions can undergo protonation and subsequent elimination of a chloro radical, in addition to the direct expulsion of a chloride ion. The results of this study and their relation to the clean-up of environmentally dangerous materials such as PCBs and dioxin will be discussed.

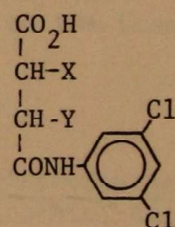
Hsien-Cheng Shih and Gary O. Rankin, Dept. of
Pharmacology, Marshall University School of
Medicine, Huntington, West Virginia 25704-2901.
Synthesis of racemic α -hydroxy- and β -hydroxy-
N-(3,5-dichlorophenyl)succinamic acid and their
cyclic imide.

N-(3,5-Dichlorophenyl)succinimide (NDPS) 1 is an experimental agricultural fungicide. NDPS has been shown to produce selective nephrotoxicity in Sprague-Dawley and Fischer 344 rats which is characterized by diuresis, increased proteinuria,

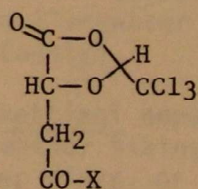
glucosuria, hematuria, increased blood urea nitrogen (BUN) concentration and kidney weight, decreased organic ion accumulation by renal cortical slices and proximal tubular necrosis. Previous studies from our laboratory have demonstrated that NDPS produces nephrotoxicity via a metabolite. This metabolite results from an oxidation reaction on the succinimide ring of NDPS. However, the identity of this metabolite is unknown. The purpose of this study was to synthesize three hydroxy metabolites of NDPS; α -hydroxy-N-(3,5-dichlorophenyl)succinamic acid (2), β -hydroxy-N-(3,5-dichlorophenyl)succinamic acid (3), and 2-hydroxy-N-(3,5-dichlorophenyl)succinimide (4) for nephrotoxicity screening. All compounds were prepared from the chloralide (5) which was synthesized from d,l-malic acid and trichloroacetaldehyde using the method of Eggerer and Grunewald (Liebigs Ann. Chem. 677:200, 1964). Pure 5 (m.p. 172-174°C) was obtained by precipitation from water in a 90% yield. Compound 5 was reacted with 3,5-dichloroaniline (DCA) in refluxing benzene to produce 3 (m.p. 130-132°C). Treatment of 5 with thionyl chloride followed by DCA yielded two products, 4 and an intermediate 6 (m.p. 94-95°C). Pure 4 (m.p. 144-145°C) was obtained from the mixture by either recrystallization from benzene or column chromatography using silica gel as the solid support and eluting with a chloroform: acetone gradient. Compound 2 was prepared via alkaline hydrolysis of 6 followed by acidification. Pure 2 (m.p. 140-142°C) was obtained using column chromatography (silica gel: chloroform: acetone: ethanol gradient). This work was supported by NIH grant DK 31210.



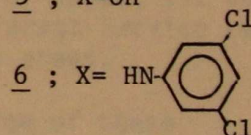
1 ; X = H
4 ; X = OH



2 ; X = OH, Y = H
3 ; X = H, Y = OH



5 ; X = OH



6 ; X = HN-

STEVEN L. STEPHENSON and TAMMY K. KELLEY, Dept. of
Biology, Fairmont State College, Fairmont, West
Virginia 26554. Use of the microcomputer in
taxonomy and ecology.

Microcomputers have many possible applications in taxonomy and ecology. Although some familiarity with the use of microcomputers is a necessary prerequisite, it has been our experience at Fairmont State that it is possible for students (and their professors!) to develop this in a remarkably short period of time. In ecology, the microcomputer can be used (along with the appropriate software) to store, sort, and analyze extensive data sets, to calculate such things as importance values, and to generate similarity and diversity indices. In taxonomy, it can be used in conjunction with a simple data storage/retrieval system to manage teaching collections or small herbaria and also (when used along with a printer) to produce specimen and/or annotation labels. Another recent (and potentially exciting) use that has been made of the microcomputer is that of constructing user interactive synoptic keys for the identification of various groups of organisms.

Ecology

CAROLE S. RYAN, WILLIAM J. VAIL and ROBERT K. RILEY, Department of Biology, Frostburg State College, Frostburg, Maryland 21532. The Role of Azygospores in the Transfer of VAM Colonization.

Root and soil analyses were utilized to determine the role of the azygospores of Gigaspora margarita in the transfer of vesicular-arbuscular mycorrhizal (VAM) infection between Bahia grass roots. Following mycorrhizal establishment in Bahia grass inoculum plants, two experiments were conducted using these plant roots. In one experiment azygospores associated with the plant roots were removed, and in the other, azygospores were allowed to remain attached. Bahia grass seeds were sown and permitted to germinate and grow with the inoculum plants. At 3 and 6 months, microscopic examination of root segments from both experiments indicated VAM colonization in experimental plants. Root and soil analyses showed azygospores present only in that experiment where azygospores were not removed. We conclude that azygospores are not required for the transfer of G. margarita infection between Bahia grass roots.

THOMAS K. PAULEY, Dept. of Biology,
University of Pittsburgh at Bradford,
Bradford, Pennsylvania 16701. Habitat Disturbances on the Cheat Mountain Salamander.

The Cheat Mountain salamander (Plethodon nettingi) is the only amphibian, and possibly the only vertebrate, known to be endemic to West Virginia. It is confined to elevations above 3,100 ft. (945 m) in an area that extends from Blackwater River Canyon (Tucker County) to Thorny Flat (Pocahontas County).

There are 54 known P. nettingi populations. Of these, all but one have been disturbed by either hiking trails, Forest Service roads, haul roads, strip mines, deep mines, or recreation areas.

Plethodon nettingi has been extirpated in three of these populations. In 1986, a ski slope was developed in Canaan Valley adjacent to one of the remaining 51 populations. A five-year study of this population will be conducted to help determine the effects of such disturbances on adjacent populations.

DENISE SCHMIDT and DONALD TARTER, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25701. Observations on the ecological life history of *Acroneuria carolinensis* from a West Virginia stream (Plecoptera: Perlidae).

The ecological life history of *Acroneuria carolinensis* (Banks) from Panther Creek, Nicholas County, West Virginia were studied from December 1983 to November 1984. Length-frequency distribution indicated a two-year life cycle. The largest naiads (26.0 mm) were collected in February, April and May. Females and males exhibited greatest growth in August (28%) and October (21%), respectively. Naiads underwent 25 instars. A chi-square test applied to 72 males and 152 females showed a significant deviation (0.05 confidence level) from the expected 1:1 sex ratio. Naiads were carnivorous in their feeding habits. Plecopterans ($\bar{X} = 1$, %FO = 15.9) and ephemeropterans ($\bar{X} = 1$, %FO = 12.5) made up the principle components of the diet. Dipterans were important components in the winter and summer, while trichopterans became important in the summer. An approximate TLM₂₄ pH value of 3.4 was determined for the naiads. A phoretic relationship between *A. carolinensis* naiads and a chironomid midge was observed on 3.7 percent of the naiads examined. Emergence was between 1 June and 16 June; peak emergence occurred on 8 June 1984. In the laboratory, emergence occurred between 9:30 p.m. and 1:45 a.m. Direct egg counts for adult females ranged from 10 to 800 eggs ($\bar{X} = 239$) per female. There was little correlation between body length and number of eggs ($r = 0.56$). Mean egg length was 0.43 mm (range 0.38-0.48) and mean egg diameter was 0.33 mm (range 0.29-0.38).

Geology and Mining Science

William C. Grady, West Virginia Geological and Economic Survey, P.O. Box 879, Morgantown WV 26507. Relationships between living organisms and sediments in the shallow nearshore environment off the Florida Keys.

The shallow nearshore environment on the Atlantic Ocean side of the Florida Keys extends from the sublittoral shoreline to approximately 2 meters water depth and 500-2000 meters offshore from the islands. Within this environment 9 subenvironments were identified based on the type and abundance of organisms. From the shoreline seaward these were: (1) Nearshore Thalassia (Turtle grass) carpet. (2) Syringodium (Manatee grass) barrens. (3) Halimeda incrassata barrens. (4) Nearshore open sands. (5) Nearshore Halimeda opuntia masses. (6) Offshore Thalassia carpets (over sediment filled sinkholes). (7) Offshore Thalassia-Goniolithon-Porites subenvironment. (8) Offshore Halimeda opuntia mounds. (9) Offshore hardground. Sediment samples collected from representatives of each subenvironment were sieved for size distribution and thin-sectioned skeletal constituent composition analysis of the sand size fraction. The silt-clay size ($<64\mu$) fraction of the sediment was greatest in the nearshore Thalassia carpet subenvironment (54%) and in the Syringodium barrens (12%) and less than 3% of the sediment in all other subenvironments. Sand-size sediment dominated (57-94%) in the low mud subenvironments. Skeletal grain analyses of the sand-size sediments averaged: 38% Halimeda fragments, 16% foraminifera, 14% mollusk fragments, 9% coralline algae fragments, and 6% coral fragments. Peloids, grains without skeletal structures because of fragmentation and microbial borings, accounted for an average 14% of the sediment. Skeletal grains most abundant in the nearshore subenvironments were Halimeda fragments (47% of the sediment) and peloids (16%). Skeletal grains most abundant in the offshore subenvironments were mollusk fragments (17%), foraminifera (21%), coralline algae (12%) and corals (7%). Further research will determine if statistical differences can be observed between the subenvironments on the basis of skeletal constituents.

STEVEN McCLELLAND and THOMAS R. JAKE. West Virginia Geological and Economic Survey, Box 879, Morgantown, WV. A Vertebrate Trackway Occurrence Above the Pittsburgh Coal (Monongahela Formation, Upper Pennsylvanian) in Mineral County, West Virginia.

Two vertebrate trackways were found in a Pittsburgh coal (late Pennsylvanian age) surface mine located $1\frac{1}{2}$ miles east of Barnum and $1\frac{1}{2}$ miles south of Warnocks in Mineral County, West Virginia. The trackways were not in place when found but they were from the interval from the roof of the Pittsburgh coal to the base of the Sewickley coal (lower Monongahela Formation.) The footprints are about 12 centimeters long and 9 centimeters wide and show the impressions of five toes. The trackway had a stride of 29 centimeters, a pace of 28 centimeters, a step angle of 75° , and a trackway breadth of 25 centimeters. The distance between the midpoint of a line between two forefoot impressions and the midpoint of a line between two hindfoot impressions is 35 centimeters.

JO DAVISON, President/Research Director; Lambda Group R & D Labs, Incorporated; 1445 Summit Street, Columbus, Ohio 43201. A new breakthrough in the microbial cleaning of slurry coal fines.

Coal is West Virginia and West Virginia is coal; these are trying times for both. Acid rain, tall stacks legislation, and uncleanable high sulfur coal have led to layoffs and increased utility rates. Nobody can agree on who's responsible for the problem or what its solution is. Lambda does not have all the answers, but proposes a new, cost-effective, easy-to-use method of cleaning the sulfur from coal fines prior to combustion. The cleaners are called I.M.P.P.S. - Immobilized Microbial Pollution Purification Systems - which is exactly what they are. Prior art in the microbial cleaning arena has included activated sludge, genetically engineered "super-bogs," and organisms from hot sulfur springs that work only when coal fines are boiled. None has achieved consistently high removal of sulfur, especially the organic sulfurs such as dibensothiophene, the most prevalent of the organics.

For microbial coal cleaning to be seriously considered on a large scale, five major problems had to be solved: (1) The "mat" of jarosite, sulfite, and others that catch in the lipophosphate covering of the bacteria bonded to the coal fines like super glue; the Lambda Process IMPPS do not form any such mats. (2) The process had to be adaptable to present coal-cleaning plant equipment; Lambda's is. (3) It had to clean a ton an hour; Lambda's takes four hours and requires a three-hour lag at the start. (4) The removed oxidized sulfur and metals had to be separated from the coal fines; the IMPPS are larger than the fines and are easily separated by dewatering screens. (5)

The process had to be cost-effective; Lambda's will cost \$10 per ton. It also has to work. In evaluations by independent test labs for the Ohio Coal Grant monies, the Lambda IMPPS were consistently found to remove 70-90% of the sulfur and ash-creating metals. The process was completed through a research grant to the alpha prototype.

E. RAY GARTON, Mammoth Geo, Inc., P.O. Box 200, Barrackville, WV 26559. The Saltpetre Caves of West Virginia: Progress Report.

Saltpetre, potassium nitrate (KNO_3), was the principal ingredient of gunpowder until the close of the Civil War. Evidence of mining for saltpetre has been reported from 44 natural, limestone caves in 9 West Virginia counties. Many of these caves were mined during the Revolutionary War, War of 1812 and the Civil War. The importance of West Virginia's saltpetre caves to these war efforts is largely unknown and reported in the historical accounts of these conflicts. During the 18th and 19th centuries saltpetre and the mining of saltpetre was central to the politics and economics of this young country. However, for all the importance of saltpetre, the exact nature of its origins and exactly how it was used in the manufacture of gunpowder is still largely unknown. Sometime during or soon after the end of the Civil War nitrates became more readily available from foreign sources or nitrogen fixation technology and the saltpetre industry in America died.

E. RAY GARTON, Mammoth Geo, Inc., P.O. Box 200, Barrackville, WV 26559 and FREDERICK GRADY, 1201 South Scott Street, Apt. 123, Arlington, VA 22204. The Paleozoic Vertebrates of West Virginia: Progress Report.

Fossil remains of Paleozoic vertebrates have been identified from 108 localities in 30 West Virginia counties. Included in the remains are the Classes Reptilia, Amphibia, Osteichthyes (bony fish) and Chondrichthyes (sharks) representing 49 genera. The age of the deposits range from the Permian Period (230 million years ago) through the Devonian Period (410 million years ago). Most of the Paleozoic vertebrates of West Virginia are known only from fragmentary remains and often cannot be identified to species. Among the reptiles represented are: Dimetrodon and Edaphosaurus cf. curciger. Amphibians represented include: Eryops cf. megacephalus, Diploceraspis

burkei, Greerepetron burkemorani, Mauchchunkia bassa, Branchiosaurus, Lysorophus dunkardensis, and Proterogyrinus scheelei. Osteichthyes represented include: Paleoniscis, Megalichthyes, Holotychius, Ectosterochachis, Sphaerolephis and the lung-fishes Tradodis casterensis and Monongahela dunkardensis. Chondrichthyes represented include: Orthocanthus, Xenacanthus, Helodus simplex, Dittodus, and Physonemus falcatus.

E. RAY GARTON, Mammoth Geo, Inc., P.O. Box 200, Barrackville, WV 26559 and FREDERICK GRADY, 1201 South Scott Street, Apt. 123, Arlington, VA 22204. The Quaternary Vertebrates of West Virginia: Progress Report.

Fossil and skeletal remains of Quaternary (Pleistocene and Holocene) vertebrates have been identified from 90 localities in 19 West Virginia counties. The majority of the localities, 77 versus 13, are cave deposits. Included in the remains are reptiles, amphibians, birds, fish and mammals representing 98 genera. The age of the deposits range from recent to perhaps 700,000 years. Among the mammals are at least 25 extinct species including: Mammuthus columbi (woolly mammoth), Mammut americanum (mastodon), Symbos cavifrons (musk-ox), Smilodon (sabertooth cat), Canis dirus (dire wolf), Panthera onca (jaguar), Tapirus (tapir), Platygonus and Mylohyus (peccary), Miracinonyx inexpectatus (cheetah), Megalonyx jeffersoni (ground sloth), Arctodus pristinus (cave bear), Equus tau (horse), and Desmodus (vampire bat). Also included are many extant species of northern, western and southern forms indicating the extreme climatic changes that occurred during the Pleistocene of West Virginia. These forms include: Sorex arcticus (arctic shrew), Synaptomys borealis (northern bog lemming), Dicrostonyx hudsonius (collard lemming), Taxidea taxus (badger), and Dasypus bellus (armadillo). Some of the taxon may represent new species while many of the taxon represent first records and range extensions for West Virginia and the Appalachians.

E. RAY GARTON, Mammoth Geo, Inc., P.O. Box 200, Barrackville, WV 26559 and FREDERICK GRADY, 1201 South Scott Street, Apt. 123, Arlington, VA 22204. and ALAN R. CARPENTER, Rt. 6 Box 72-J, Fairmont, WV 26554. Mastodon, Mammut americanum, Remains Recovered From Big Springs Cave, Tucker County, West Virginia.

A single tooth cusp of the extinct Pleistocene Proboscidean Mammut americanum (mastodon) was recovered from Big Springs Cave, Tucker County, West Virginia. The discovery represents the first record of mastodon from Tucker County, the 14th record for West Virginia, extends the geographic range of mastodon in West Virginia, and represents the greatest altitude (2450 feet, 747 meters) of mastodon yet discovered in the State. The mastodon became extinct near the end of the Pleistocene about 10,000 years ago. Remains of porcupine, Erethizon dorsatum, were also recovered from the cave. This porcupine became extinct in West Virginia during recent times.

EBERHARD WERNER, P. O. Box 795, Morgantown, WV 26507. **Aeromagnetic lineaments of West Virginia.**

The aeromagnetic map of West Virginia shows several prominent lineaments. Axes of high and low magnetic field strength were drawn in a manner analogous to drawing synclinal and anticlinal axes on a structure contour map. The resulting patterns show textural boundaries, where the general nature of the arrangement of the axes undergoes considerable change. The most obvious change occurs along a line trending west-southwest to east-northeast, at approximately the location where the 38th Parallel Lineament has been delineated by structural and photogeologic data. To the south of this boundary, the magnetic axes can be characterized as chaotic; to the north, they are quite straight and regular and essentially parallel to the general Central Appalachian trends. Less obvious lineaments are delineated by inflections and discontinuities of the axes. The most prominent of these are zones trending northwest to southeast; one of these coincides with Woodward's "Cross Appalachian Fault Zone." For the most part, the magnetic lineaments probably reflect basement variations of both depth and composition. Although reduction of iron by escaping hydrocarbons has been suggested, effects so produced are probably of too small a scale to be shown in the aeromagnetic data of the state map. Some of the magnetic lineaments are also reflected by gross stratigraphic patterns; this probably indicates basement tectonic activity throughout the deposition of the Paleozoic rocks.

Psychology

ALAN CHRISTOPHER and THOMAS CRITCHFIELD
West Virginia University
Gambling: A contextual analysis

Traditionally, gambling behavior -- particularly compulsive or chronic gambling -- has been viewed within the framework of a medical model which assumes the behavior to be a manifestation of internal disease. An alternative but relatively undeveloped perspective is that gambling results from specifiable events within the context of an individual's past experiences and current situation. Basic operant research has shown that behavior which produces variable consequences ("risk-taking") can be more persistent, and more preferred, than "safe" behavior with predictable, constant payoffs. This paper outlines a model of gambling behavior based on the operant choice paradigm and juxtapositions of variable probabilities and magnitudes of reinforcement. Data are presented suggesting that gambling-like behavior can be produced in both humans and infrahumans by manipulating reinforcement parameters in choice situations. The implications of such a model, if valid, range from the creation and regulation of games of chance to possible insights for the treatment of chronic gambling.

THOMAS CRITCHFIELD, KARLA DOEPKE and TIMOTHY FREEMAN
Institute for Behavioral Studies, Morgantown,
WV 26506
Rate-based mastery: Cross-modal transfer in
basic academic skills

The movement known as Precision Teaching has emphasized the dissection of academic skills into discrete, countable responses with fluency defined in terms of the ability to emit correct responses at very high rates. While the method as a whole is demonstrably effective, one assumption of Precision Teaching that remains to be tested is the notion that high-rate mastery produces generalization of skills to new situations and/or new modalities of information exchange. This study consists of a parametric analysis of the relationship between rates of correct responding on a training task in one modality and success on related but untrained tasks in other modalities. Preliminary results indicate different patterns of transfer for different students, and the possibility that transfer patterns may be predicted by student proficiency on certain "building block" skills. Implications for individualized instructional strategies are discussed.

MATTHEW P. HUSHIN and JOHN H. HULL,
Dept. of Psychology, Bethany College,
Bethany, West Virginia 26032. Effects
of cartoons on attitudes toward and
retention of a textbook passage.

Female and male college students read textbook passages about socialization theories, then were measured for retention of the material and for attitudes about the material. Half the males and half the females read passages containing two "Dennis the Menace" cartoons related to socialization; other subjects' passages contained no cartoons.

Separate 2 (subject gender) by 2 (cartoon presence or absence) analyses of variance for retention and attitude measures showed: no significant differences on retention; females significantly more than males reported wanting to study the topic in depth in a class; males without cartoons in their passages rated the passage significantly duller and significantly less enjoyable than did males with cartoons in their passages. Results may depend in part upon the type of cartoon used in the passage.

KARL D. FEZER, Dept. of Biology, Concord College,
Athens, West Virginia 24712. To Which Questions
Raised in the Creation/Evolution Controversy
Should Public Schools Give Definite Answers?

The creation/evolution controversy encompasses many disputed claims. Of the most fundamental questions, all are worthy subjects of discussion, but only three are such that a definite answer is both justified and important for the integrity of science education. These three are: Can explanations invoke supernatural concepts and still be scientific?, How old is the earth?, and Do diverse organisms share common ancestors? Questions that do not meet the criteria are: How should Scripture be interpreted? Is theism (or atheism) rational? Must supernaturalism be invoked to make nature intelligible? Did life arise and then evolve through natural processes exclusively?

KENNETH H. BAKER, Dept. of Biology,
Concord College, Athens, West Virginia
24712. Creation/Evolution as a Conflict
Between Two World Views

It may be an expression of a sincere desire to avoid controversial issues that prompts some to ignore THE central question in the creationism/evolution debate. The fundamental premise of "scientific" creationism is that a supernatural creator (i.e. God) exists. Just as Paley's Argument From Design implies a Designer, creationism requires a Creator. Any claim creationists make is some form of "God did it!" or, "God made it that way!".

Since the basic assumption of creationist beliefs is that God is real, it would seem that the only thing necessary to discredit creationism is to demonstrate that the God hypothesis is scientifically untenable. (or as Sidney Hook has suggested, "a speculative hypothesis of an extremely low order of probability.")

Sociology/ Anthropology/Archeology

JOHN R. WARNER, JR. Department of Sociology and Anthropology, West Virginia Wesleyan College, Buckhannon, WV 26201. The revolution in Juvenile Justice in West Virginia 1977 - 1985. A revised paper.

As part of a series of papers on rural crime and delinquency presented before this Academy by the author, this is a revision of a paper presented in 1983 which covered the years 1977 - 1985. The paper presented here updates the 1983 report. No further revisions are intended, as the "revolution" seems to have spent itself by the end of 1985. It seems appropriate to tie it all together and to view that revolution from beginning to end.

The paper develops the argument that West Virginia responded to the federal incentives provided by the Juvenile Justice and Delinquency Prevention Act of 1974 (1977, 1982) in a vigorous and unique manner, and that the results of that response place West Virginia in the forefront of juvenile justice.

JOHN R. WARNER, JR., Department of Sociology and Anthropology, West Virginia Wesleyan College, Buckhannon, WV 26201. The early history of the State Penitentiary at Moundsville, West Virginia 1866 - 1900.

As part of a series of reports on rural crime presented before this Academy by the author, this paper is a social history of the early years of the West Virginia State Penitentiary at Moundsville.

Years covered in this report are from the origin of the prison in 1866 through the end of the Nineteenth Century. The method of research has been an analysis of the biannual prison reports submitted by the Board of Directors to the Governor.

The paper focuses on the reports of the Board, the Superintendent, and the Medical Officer of the prison, raising those issues which appear to be critical to the administration of the prison at the time. Among those issues are the construction of the prison, prison labor, an adequate supply of water, causes of death and disease, making the prison economically self-sufficient, reducing the cost of feeding the prisoners, overcrowding, issues of prison management and discipline, theories of rehabilitation and reform, the use of violence and torture by the guards, escapes and violence initiated by the prisoners themselves.

The argument is made that the current problems faced by prison officials can be better understood in the light of an adequate knowledge of the historical context of many perennial prison problems.

Richard S. Little, Dept. of Geology
and Geography, West Virginia 26506.
Evacuation Planning in West Virginia.

This history of Evacuation Planning in West Virginia is sketchy. With the advent of new computer assisted simulations emergency managers can maintain a higher level of preparedness. Hancock County, as part of the Beaver Valley Nuclear Power Evacuation Zone, has an existing simulation system. The Kanawha and Ohio Valley chemical complexes and their associated transportation networks present a high of special concern.

The emergency evacuations of Buffalo Creek and portions of the Cheat River and South Branch Rivers during disastrous floods provide a scenario for future planning and preparedness. All elements of society are involved with special responsibilities on the elected officials and the perceptions of the average citizen.

Local standards of readiness are generally low in West Virginia, despite a deadly history of flooding and a growing frequency of toxic material releases.

ORVILLE R. GURSSLIN, Dept. of Sociology, Ohio University, Athens, Ohio 45701 and JOHN D. RICHARDS, Dept. of Sociology and Philosophy, West Virginia State College, Institute, West Virginia 25112. On the universality and necessity of social hierarchies.

In this paper we examine the arguments for the universality and necessity of social hierarchies generated from both hierarchy theory and organizational theory in sociology. According to hierarchy theory hierarchies are universal in that they are the inevitable result of the evolutionary development of all complex systems. Further, hierarchies are not only considered as being the most efficient arrangement of complex systems; they are regarded as being functionally necessary for the operation of complex systems as well. Another characteristic usually attributed to hierarchies is that they exist on both a descriptive and structural level.

Although most theorists would agree that there is a distinction between natural, physical hierarchies and artificial social hierarchies they attribute the same general characteristics to both. However, it is our contention that this distinction is more than just a heuristic one. By offering evidence of the existence of non-hierarchical human organizations we propose that social hierarchies are neither universal nor functionally necessary. This evidence leads us to question the efficiency of hierarchy in organizations. Further, since social hierarchies are artificially imposed on organizations, we conclude by examining some possible reasons of why social hierarchies exist, and how they are perpetuated.

In the final section of our paper we present a possible explanation for the widespread presence and perpetuation of social hierarchies. In light of our findings we contend that social hierarchies are artificially imposed on organizations and perpetuated by the elite of a society as a means of maintaining control in order to preserve its power and protect its interests.

H. MICHAEL DORSEY, West Virginia Department of
Highways, 1900 Washington Street, East,
Charleston, West Virginia 25305, Rockshelter
Excavations in Nicholas and Webster Counties,
West Virginia.

During September 1982 and August 1983, test excavations were performed at three rockshelters in the southwestern section of the Monongahela National Forest in Nicholas and Webster Counties, West Virginia. A single, one by one meter test excavation unit was placed in each of the shelters in an attempt to identify the cultural periods represented in the numerous sites of this type located in this area of the state.

Cultural materials were recovered from each of the sites, although varying degrees of disturbance limited the amount of information that could be retrieved from the sites. One test unit was heavily disturbed by rodent burrowing, another was largely obscured by a rock fall and the third provided a relatively clear stratigraphic record.

Problems of interpretation are present, even for the relatively undisturbed site, because of a lack of corroborating data from similar undisturbed sites in the area. The heavily disturbed site points to a problem in accepting data from previous work in that the degree of disturbance is frequently unknown, though some disturbance is usually mentioned in early reports.

ROBERT F. MASLOWSKI, Planning Division, U.S. Army Corps of Engineers, 502 Eighth Street, Huntington, West Virginia 25701. Relationship of archeological sites to SCS soil types in West Virginia.

Soil Conservation Service soil series have proven to be the most significant variable for predicting the locations of archeological sites. Elevation, slope, relationship to water, drainage, soil productivity and acidity, important attributes for site prediction and interpretation, are incorporated into SCS soil series definitions. Modern SCS soil maps available for West Virginia counties are used to develop Site/Soil Indices which are useful in predicting locations of archeological sites. These Site/Soil Indices will be useful in planning future archeological surveys, determining where deep testing is appropriate and determining the adequacy of archeological surveys.

W. HUNTER LESSER and JANET G. BRASHLER, Monongahela National Forest, Elkins, West Virginia. Can we go beyond site distribution? Cultural models and lithic scatters from the eastern West Virginia uplands.

Problems in the interpretation of upland lithic scatters based on six years of archaeological site survey and test excavations on the Monongahela National Forest are reviewed and compared with adjacent areas. Settlement models previously described for the uplands are discussed and a derived model based on testing of selected lithic scatters is presented. However, it is argued that settlement distribution models are limited in scope and useful primarily as predictors of site locations. Directions for current and future research on lithic scatters are discussed.

GLORIA GOZDZIK, Institutional Analysis and Planning, West Virginia University, Morgantown, West Virginia, 26506. Ethnoarcheology: Trend or Fad?

All archeologists have been faced with problems of interpretation. Stone tools, bones, pot sherds, fire pits and structures are the "stuff" of archeology but the interconnection of all the technological aspects of an archeological site are only one aspect of a culture. Although no one can "dig-up" a religion or a residence pattern the archeologist is constantly faced with the task of putting anthropology into archeology. The ethnoarcheologist has taken this delima and tried to reconstruct past history using old approaches in

new ways. This paper will address what ethnoarcheology is, how it has been used in the past and the possible use for it's application in the future. Examples from various archeological sites will be presented and a summary of the advantages and disadvantages will be discussed.

Zoology

E. RAY GARTON, Mammoth Geo, Inc., P.O. Box 200, Barrackville, WV 26559 and FREDERICK GRADY, 1201 South Spring Street, Apt. 123, Arlington, VA 22204. The Vertebrate Cave Fauna of West Virginia: Progress Report.

Living vertebrates have been identified from 104 localities in 16 West Virginia counties. Included in the fauna are amphibians, reptiles and mammals representing 17 genera. Among the amphibians are toads, frogs and several species of salamanders including the West Virginia Spring Salamander, Gyrinophilis subterraneus. This species is only known in North America from General Davis Cave, Greenbrier County. The only reptile represented is the rat snake, Elaphe obsoleta. The mammals represented include the woodrat, Neotoma floridana and several species of bats. Among the bats are the endangered species Myotis sodalis (indiana bat) and Plecotus townsendii virginianus (virginia long-eared bat).

MARK SHERIDAN and DONALD TARTER, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25701. Food habits of the freshwater drum, *Aplodinotus grunniens*, from Gallipolis Locks and Dam, Ohio River.

Food habits of freshwater drum, *Aplodinotus grunniens* (Rafinesque), from the Ohio River at Gallipolis Locks and Dam, were determined in October and November 1981 and from March through November 1982. Drum were found to feed most intensively in the summer. Regression analysis of the number of stomachs with food and seasonal water temperature exhibited a high positive correlation ($r = 0.80$). Fishes were the most important food item, both by weight (80.2%) and frequency of occurrence (90.2%). The emerald shiner, *Notropis atherinoides* (Rafinesque), was the primary fish consumed during the study with the gizzard shad, *Dorosoma cepedianum* (Lesueur), becoming more important as the year progressed. Aquatic invertebrates, mainly the trichopterans *Hydropsyche orris* and *Cheumatopsyche* sp., served as secondary food sources in the drum's diet. Crayfishes, *Corbicula* clams, and terrestrial invertebrates were of minor importance. Food habits of adult drum varied little with either season or size, but young-of-the-year (8.7-13.3 mm) subsisted exclusively on aquatic invertebrates, mainly unidentified copepods.

JOAN E. BURTON and RALPH W. TAYLOR, Dept. of Biological Sciences, Marshall University, Huntington, WV 25701. The reproductive cycle of *Uniomerus tetralasmus* (Say, 1831).

A population of a midwestern naiad, *Uniomerus tetralasmus* was discovered in West Virginia in 1984. A survey of this disjunct population in a small farm pond in Scott Depot was undertaken between May and December 1986. A study of the reproductive and early developmental stages was the primary objective.

When permanent slides were made of the naiad's reproductive organs, it was found to be a functional hermaphrodite. Eggs and sperm were found to be mature simultaneously and in proportions indicating the possibility for self-fertilization. Gravid "females" were found only in May suggesting that these are bradytachtic or "short-term breeders." This is a significant discovery in that less than four percent of all described species are known to deviate consistently from a strictly dioecious condition.

JAMES E. JOY, Department of Biological Sciences, Marshall University, Huntington, West Virginia 25701. *Chaetogaster limnaei* (Annelida: Naididae) in three species of aquatic snails from McClintic Wildlife Station (Mason County) ponds.

Chaetogaster limnaei was recovered from 157 of 206 (76.2%) *Helisoma anceps*; 34 of 71 (47.9%) *Physa* sp.; and 24 of 63 (38.1%) *Lymnaea* (*Pseudosuccinea*) *columella* over a nine month period (March through November 1984). A three way Chi-Square contingency test of prevalence rates verified that *H. anceps* was the "preferred" host species while *L. (P.) columella* was the least desirable host species. Differences in mean intensity levels of 4.9, 3.8, and 5.3 for *H. anceps*, *Physa* sp., and *L. (P.) columella*, respectively, were not significant. Regression analyses revealed positive correlations between size of infected host and number of *C. limnaei* individuals present for all three host species, however, only one regression curve--that plotted for height of *L. (P.) columella* versus number of *C. limnaei* individuals--was significantly different from zero.

RALPH W. TAYLOR, Dept. of Biological Sciences,
Marshall University, Huntington, West Virginia
25701. New distribution records for West Virginia
slugs (Mollusca: Pulmonata).

Little attention has been given to the distribution of slugs in West Virginia. This paper presents information on additional county records for five native species and first occurrence state records for three imported species. The native species and their additional county records are as follows: Philomycus virginicus Hubricht, (Cabell, Wayne); Philomycus carolinianus (Bosc), (Cabell, Wayne); Megapallifera mutabilis (Hubricht), (Cabell, Wayne); Deroceras laeve (Muller), (Cabell, Summers, Wayne); Pallifer dorsalis (A. Binney), (Mason).

The imported species and the counties from which they were recorded are: Deroceras reticulatum (Muller), (Cabell); Limax maximus (Lamarck), (Cabell); Lehmannia poirieri (Mabille) [= Limax marginatus Muller], (Cabell).

Abstracts of the First West Virginia Water Research Information Exchange Conference

JOSEPH M. BIFULCO, JAMES J. SHIREY, JR., and GARY K. BISSONNETTE, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506-6057. Bacteria of public health significance in rural groundwater supplies.

A bacteriological survey of untreated, individual, rural groundwater supplies was performed on 70 water systems in Preston County, West Virginia. More than 55% of the water supplies contained total coliform densities in excess of the EPA maximum contaminant level (MCL) of one CFU per 100 ml. In 32% of the water supplies, fecal coliforms were absent in the presence of total coliforms, indicating that some of the coliform contamination was of nonfecal origin. Approximately 30% of the water systems contained either fecal coliforms (FC) and/or fecal streptococci (FS). The FC/FS ratio suggested 15 of 40 water supplies (37.5%) were contaminated with bacteria from animal excrement. Since limitations exist with the use of the FC/FS ratio, implications concerning the source of bacterial pollution should be judged with caution. Mean recovery for heterotrophic plate count bacteria was 204 CFU/ml, ranging from <1 to 1780 CFU/ml. The opportunistic pathogen Acinetobacter calcoaceticus was present in more than 35% of the water supplies. In 16% of the water systems, A. calcoaceticus was isolated in the absence of total coliforms. Water supplies that were shallower, older, and lacking adequate casing characteristically contained higher densities of indicator bacteria than those which were deeper, of more recent construction, and sufficiently cased.

JOSEPH P. CALABRESE and GARY K. BISSONNETTE, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506-6057. Enhanced recovery of acid mine water-stressed Escherichia coli on media containing catalase or sodium pyruvate.

Escherichia coli (EPA 00244) 6-h cultures were exposed to filter sterilized acid mine water (AMW) and assayed for catalase activity. Significant reduction ($p < 0.05$) in enzyme activity was observed in acid stressed cells (<1.0 Units/mg) as compared to nonstressed cells (23.4 Units/mg). Resuscitation studies were conducted using non- and catalase-amended (500-2000 Units/plate) tryptone glucose yeast extract (TGY) agar. Enhanced recovery ($p < 0.05$) of AMW-stressed cells was obtained on TGY agar containing 1000-2000 Units catalase/plate

($\bar{x} = 1.4 \times 10^5$ CFU/ml) as compared to the non-amended medium ($\bar{x} = 4.2 \times 10^3$ CFU/ml). Studies were then conducted utilizing non- and catalase-amended (1000 Units/plate) nonselective medium (TGY) and two selective media (mFC and mT7). In each case, media containing catalase provided better recovery ($p < 0.05$) of stressed cells as compared to non-amended media. Supplementation of TGY agar with sodium pyruvate (0.005-2.0%) similarly resulted in improved detection ($p < 0.05$) of AMW-stressed cells ($\bar{x} = 1.9 \times 10^5$ CFU/ml) as compared to recovery on TGY agar lacking pyruvate ($\bar{x} = 3.5 \times 10^3$ CFU/ml). Studies were then conducted utilizing non- and sodium pyruvate-amended (1.0%) TGY, mFC, and mT7. Without exception, enhanced recovery (> 1.0 log increase) was observed on the pyruvate supplemented media. These data indicate that AMW-stressed E. coli may benefit from the addition of catalase or sodium pyruvate in both selective and nonselective recovery media.

L. C. COOK, P.E. and R. A. MATHEWS,
Environmental Consultant, 9825 Perry
Highway, Wexford, PA 15090. Research Analog
LTD Inc., Suit 220, 9825 Perry Highway,
Wexford, PA 15090. Wastewater Management -
Innovative Onsite Approaches.

Wastewater treatment has become an increasingly more difficult area of operations management for businesses, authorities, and developers. The rising costs of plant expansion and new construction have focused extreme pressures on operations management teams to develop cost-effective means to maximize the investment value of wastewater treatment facilities to their service areas. The migration of the American public away from large cities coupled with increasingly stringent government regulatory standards to obtain operating permits has placed flow levels into treatment facilities at or near plant capacities which were not anticipated to occur for many years. Fortunately, creative combinations of new technology and the creative application of these technologies by enterprising organizations have led to the development of Innovative Onsite Approaches to wastewater treatment permitting requirements.

JO DAVISON, President/Research Director; Lambda Group R & D Labs, Incorporated; 1445 Summit Street, Columbus, Ohio 43201. Restoration of acid-polluted aquatic systems using a new microbiological technology.

The acidification of aquatic systems by acid mine drainage, industrial and agricultural run-off and/or effluent and acid deposition is a major ecological problem world-wide. "Band-aid" solutions of dumping lime, building shallow sphagnum or cattail bogs, and spending billions of dollars to "study the problem" have done little to return aquatic systems to productivity.

The Lambda Group, Incorporated, R & D Environmental Microbiology Lab in Columbus, Ohio, has developed a significant breakthrough in the use of microorganisms to address all the above-stated scenarios in both short-term and long-range testing and scale-up. The microorganisms can be custom-grown, based on the degree of pollution and the metals present. But in all cases, the process employs mixotrophic, synergistic, symbiotic microecosystems to chelate and oxidize the pollutants and add "pioneer plants" to start succession in barren acid-destroyed water systems.

The organisms consist of bacterial groups, members of the mastigophora and ciliata groups, and chlorophyta and chrysophyta species. The key that makes them effective is a new nutrient mix that envelopes the microecosystems, forming controllable cleaning units. Their cleaning rate to date has been successful removal of 90-100% of the sulfur, iron, manganese, and aluminum in a cleaning time of four hours to five days. The result is a healthy algal growth and the beginning of second stage succession.

ROBERT N. ELI and MOO-KAB CHUN, Dept. of Civil Engineering, West Virginia University, Morgantown, WV 26506. Flood Hazard Mitigation Planning in the Cheat River Basin.

The November 4-5, 1985 catastrophic flood along the Cheat River produced peak discharges on the order of 100% higher than any in the historical records. Lives were lost and millions of dollars of damage were sustained. The flood occurred with unusual swiftness and violence that literally ripped homes and other structures from their foundations. Velocities and depths were so high that both USGS gaging stations along the main stem of the Cheat River were destroyed early in the rising phase of the flood event. Following this flood experience, public awareness was heightened and new demands for action were made. A cooperative action involving West Virginia University, Cheat Basin residents, and the Governor's office was initiated for the purpose of investigating the feasibility of providing flood protection along the Cheat River and points downstream.

In a unique effort, local governmental bodies and private citizens were given an opportunity to have a major influence on the direction of the engineering study through monthly meetings held locally during the six month duration. The procedure was to first explain the technical constraints typically found in a planning study of the sort being attempted. Within these technical constraints, local leaders and citizen representatives were encouraged to set priorities and make major decisions concerning the direction of the study. This procedure resulted in the November, 1985 flood being used as the design standard against which proposed flood mitigation alternatives were to be tested. A hydrologic model based on the U.S. Corps of Engineers HEC-1 model was fitted to the Cheat Basin for the November, 1985 event. The peak discharge at Parsons, WV was found to be approximately 180,000 cfs (cubic feet per second), increasing only slightly to approximately 205,000 cfs at Lake Lynn, PA. Although the original design of the Rowlesburg Dam would have provided good flood protection to all areas downstream, it was still strongly opposed by those living upstream. Other proposed projects involving reservoir construction, such as the Davis Power Project, were found to be ineffective for flood control. Local protection projects such as levees and flood walls were also found to offer no significant protection. Ultimately, several moderate sized reservoirs placed upstream of Parsons were found to offer good protection to all populated areas downstream. Final recommendations called for one reservoir on the Shavers Fork tributary, while two were to be placed in series on the Dry Fork tributary.

HUSSEIN GHAZI, WORAPOT CHOBTHUM, ROBERT KEEFER,
and RABINDAR SINGH, Div. Plant and Soil Sci.,
West Virginia University, Morgantown, West
Virginia 26506. Leachate analysis from three
mine spoils as affected by fly ash amendment and
cropping.

Growing concern for groundwater and stream contamination with inorganic pollutants from abandoned mine spoils and also those reclaimed with urban and industrial wastes prompted this greenhouse pot study. The objective was to examine the inorganic composition of leachate from mine spoils being reclaimed with fly ash. Three extremely acid WV mine spoils in this study were secured from Valley Point, Lenox, and Westover. The initial pH of these mine spoils varied between 3.3 to 3.8 and total S varied between 0.17 to 0.24%. The mine spoils were high in exchangeable Al and Mn. Five successive crops, i.e., two corn and three alfalfa, were grown on the mine spoils treated with fly ash at rates ranging up to 67%. Each pot had a drainage hole with plastic tubing which was connected with a 125 ml plastic bottle for leachate collection. Leachate was collected during and after the growth of each crop. Application of fly ash increased pH, EC, Ca, K, Mg and Na concentrations and decreased Al, Cu, Fe, Mn, and Zn concentrations in the leachate. Phosphorus concentrations was not affected. Concentrations of all elements in

the mine spoils decreased with each successive cropping. It was concluded that leachate from fly ash which may be used to reclaim extremely acid mine spoils should always be carefully monitored.

THOMAS R. JAKE, West Virginia Geological and Economic Survey, P.O. Box 879, Mont Chateau Research Center, Morgantown, West Virginia 26507-0879. Geology, mining, and coal reserves in the Whiteday Creek watershed, Monongalia, Marion, and Taylor counties, West Virginia.

Whiteday creek is a high-quality, cool water stream located in north-central West Virginia. It drains approximately 32.4 square miles, and flows directly into the Monongahela River near the Opekiska lock and dam at Lowsville, West Virginia. Mappable units within the basin are all Pennsylvanian in age, and include Monongahela Formation, Conemaugh Group, Allegheny Formation, and Pottsville Group sandstones, siltstones, shales, coals, limestones, and clays. Because of the pristine nature of this watershed, environmental restrictions have been strictly applied and mining has been severely limited. Recent studies indicate that 264.3 acres have been disturbed by surface mining, representing 1.3% of the surface area of the basin. Field studies have identified 43 underground mine openings within the Whiteday Creek study area. The extent of past underground mining is unknown, but not believed to be large in total area. All underground mines are abandoned. Remaining coal reserves include five seams, the Sewickley and Pittsburgh coals (Monongahela Formation), and the Upper Freeport, Upper Kittanning, and Lower Kittanning coals (Allegheny Formation). The total remaining coal reserve base in the Whiteday Creek watershed is estimated to be 154,000,000 short tons.

J.A. MARSHALL, E.C. KELLER, JR., D. WERNER, and J. FORTNEY. Dept. of Biology, West Virginia University, Morgantown, WV 26506. Preliminary Findings on Fish Distributions and Condition in Four Impacted/Non-impacted Stream in WV.

Impacted and non-impacted sites in four West Virginia streams viz., Hominy Creek in Nicholas County, Laurel Creek in Fayette County, Meadow Creek in Summers County, and Tenmile Creek in Harrison County were surveyed. Several fishery parameters were examined and four of those parameters (fish distribution, biomass, condition, and diversity) are presented herein for the stream surveys of the fall of 1986. The impacted sites were all due to mine drainage. These sites were neither highly acid nor alkaline and all had approximately neutral pH levels.

No apparent differences in species distribution exists for Laurel Creek, Hominy Creek, or Tenmile Creek although occasionally species present in one section of the stream were not represented in samples from the other collection site. Nevertheless, in most cases the same species was found in both impacted and non-impacted samples. Meadow Creek, on the other hand, showed a severe reduction in the number of species present in the impacted zone. This can probably be attributed to some extent to a low water level at the chosen sampling site.

When biomass was compared between zones, it was found that some species showed increases in biomass and some decreases in biomass in the impacted zones. In Laurel Creek, four species showed an increase and four a decrease. In Hominy Creek three species showed an increase and two a decrease. In Tenmile Creek however, only three of the 11 species showed an increase in biomass in the impacted zone. For Meadow Creek, because of the lack of species, comparative estimates cannot be made.

There was essentially no difference between condition factors for fishes from impacted and non-impacted areas of Laurel and Hominy Creeks. For Tenmile Creek, the average difference in condition factor for the 11 species common to both impacted and non-impacted samples was 0.493. Seven out of the 11 species occurring at both sites had differences in condition factors of 0.5 or higher. Meadow Creek had only one species in common for a condition factor difference of 0.286.

Species diversity was similar between the impacted and non-impacted sites in Laurel Creek, Hominy Creek, and in Tenmile Creek. In Meadow Creek a large difference in diversity existed because of the lack of fish in the impacted zone. However, this may be due to the low water level rather than any inputs coming from the drainage area.

MICHAEL R. MILLER and JAMES B. BLAIR

Department of Biochemistry

West Virginia University

Morgantown, WV 26506

Preparation of Isolated Fish Hepatocytes for
Studying Carcinogenesis

Interest has heightened in the use of teleosts for bioassay testing, detection of carcinogens in the environment, and as potential comparative oncology models for human cancer. The liver is especially sensitive to carcinogens in fish and higher vertebrates. Isolated trout hepatocytes are being studied to better evaluate the potential of this animal as a useful test system. Isolated hepatocytes are prepared by perfusing the liver with collagenase. Optimum conditions for maintaining the cells in culture are being examined. The cells do not adhere well to plastic culture dishes. However, the hepatocytes attach quickly to plastic dishes coated with trout collagen and remain attached and viable. The normal morphology of trout hepatocytes is maintained on collagen coated plates. When isolated trout hepatocytes are exposed to some DNA damaging agents, DNA repair is induced. The DNA repair efficiency and capability of the hepatocytes is being characterized to learn more about the response of trout cells to xenobiotics and to possibly serve as a means of detecting genotoxic compounds.

C. Russell Radcr
Office of Environmental Health Services
1800 Washington Street, East
Charleston, WV 25305
Alternative Individual Sewage Disposal
Systems: A Method of Protecting Groundwater

A number of private and governmental agencies have expressed concerns that the use of on-site soil absorption systems increases the degradation of the groundwater. A number of experimental systems have been used on existing homes to correct health hazards and prevent well (groundwater) contamination. The experimental systems are, but not necessarily limited to, evap-transpiration systems, recirculating and intermittent filters, and home aeration units. The evap-transpiration systems appear to be working well in West Virginia. While in the infancy stage, the results from home aeration units recirculating and intermittence filters are products high equality of effluent.

MAJID RASOUI, Chemical Engineering Department,
West Virginia Institute of Technology, Montgomery,
WV. Finger-printing Brines of West Virginia.

Contamination of groundwater by saline solutions is most often associated either with brines produced during oil and gas production operations or with the use of road de-icing agents. Expeditious identification of the source of such contamination is necessary to design appropriate remedial measures. Selected ionic ratios, namely Ca/Mg, Na/Mg, Mg/K, Ca/K, and Cl/Mg, can be used quite efficiently to distinguish road de-icing salts from brines, as contaminants in groundwater. In November 1986, WV Institute of Technology's Chemical Engineering Dept. started a one year survey for WV-DNR and U.S.EPA consisting primarily of collecting and analyzing brine samples from oil and gas fields of West Virginia. The primary objective of the report is to obtain and characterize the inorganic chemical constituents in brines.

DONALD WERNER, E.C. KELLER, JR., J.A. MARSHALL,
and JAMES FORTNEY. Department of Biology, West
Virginia University, Morgantown, WV. Preliminary
Modeling of Fish Populations in Five Streams with
Mine Drainage at Slightly Alkaline pH Levels.

Preliminary modeling efforts were completed on information obtained from two data bases from five mine drainage streams in West Virginia. One data base was obtained from a WVDNR project and the other from a WWRI project. Data were available for phytoplankton, fish, benthic invertebrates, and chemical/physical attributes. Stepwise regression analyses were performed on independent (chemical/physical) and on dependent (biological) indices which were constructed from the major factors obtained from factor analyses done on the combined WWRI and WVDNR data bases. The factors used were those which accounted for the greatest amount of variation in the data as grouped by category (i.e., chemical/physical, phytoplankton, benthic invertebrate, or fish). An overall schema of the interrelationships among all indices was prepared by the use of linear correlations among all indices. The schema shows both the nature and the degree of relationship obtained.

The preliminary model indicated that the fish in Hominy Creek (which is characterized by high numbers of species and fish with some fish preferring pools and others preferring riffles --- fish index #1) was primarily related to the variation in the oxygen/temperature index. Also, dissolved materials was related to this primary fish index. The fish index of another location (fish index #2) has fish primarily found in Mud River (which is characterized by slow moving warmer waters) is also highly related to the oxygen/temperature complex and is only indirectly related to the dissolved materials index (possibly through two invertebrate indices). The lesser of the three fish indices (fish index #3) (in terms of the variation accounted for) is dominated by the fish of Mud River at site 1 (characterized again by slow moving warmer waters, but with some habitat difference --- Fish index #3) is also related to the oxygen/temperature complex and is, again, possibly related indirectly with two benthic invertebrate indices.

Clearly, two aspects dominating this preliminary model are: 1) the dominance of Mud River data since it was considerably different from the other streams, both in terms of water chemistry and in terms of fish species and 2) by the primary chemical/physical index (oxygen/temperature) which is undoubtedly some aspect of seasonal changes.

EBERHARD WERNER, P. O. Box 795, Morgantown,
WV 26507. **The West Virginia Speleological Survey as
a source of water-related information.**

The flow of water, below and above ground, in areas influenced by karst is often radically different from that of other areas. Many geologists and engineers are not particularly familiar with the special problems of karst terrains. Several instances of karst-related problems encountered during construction in West Virginia in recent years were made more severe than necessary because of this lack of familiarity. Information on the West Virginia karst, especially that developed on the Mississippian Greenbrier Group of limestones, is not widely disseminated in the literature typically encountered by those whose responsibility includes designing and monitoring major construction projects. The most common water-related problem associated with such projects is the muddying of springs feeding fish hatcheries and small public and private water supplies. Knowledge of the recharge areas and subterranean water paths feeding such springs could reduce the frequency or severity of such problems.

The West Virginia Speleological Survey is a loosely organized group which has collected a considerable body of information on West Virginia's karst areas. Information on subterranean water flow is probably the most useful to those outside the karst research community. Most subterranean water path traces through cave conduits of the State have been performed by members of the organization. Caves and springs of the karst region have been catalogued and data pertaining to them, such as spring source areas, have been gathered. Most of the data are not published in mainstream geological literature, although the Speleological Survey does produce some specialized, low-circulation publications incorporating some of these data. The Speleological Survey maintains a few data files, but mainly serves to generally coordinate research projects in the karst and to disseminate information on the availability and location of West Virginia karst information. (At the date of this writing the mailing address of the WVSS is P. O. Box 200, Barrackville, WV 26559.)

**PROCEEDINGS OF THE WEST VIRGINIA
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