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SOCIETY FOR INVERTEBRATE PATHOLOGY EDITOR: JOHN C. HARSHBARGER

VOL. II, NO. 2 JULY 7, 1969

SOCIETY FOR INVERTEBRATE PATHOLOGY MEETING WITH THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES AT THE UNIVERSITY OF VERMONT, BURLINGTON, VERMONT, AUGUST 17-22, 1969

GENERAL PROGRAM

August 17. Evening Executive Council Meeting.
[Terrill Home Economics 120]

August 18. Morning. AIBS Plenary Session.

1:30-4:00 p.m. Contributed Papers, Session 1.
[Terrill Home Economics 108]

Evening: AIBS Bar-B-Que

August 19. 9:00-12:00 a.m. Contributed Papers, Session 2.
[Terrill Home Economics 108]

1:30- 4:00 p.m. Contributed Papers, Session 3.
[Terrill Home Economics 108]

6:00 p.m. No Host Cocktail Party
Society's Annual Banquet (Purchase tickets at the Registration Table on a "first come, first serve" basis.
[Colony Room of the Hotel Vermont]
Dr. A. K. Sparks, Presidential Address: "The Future of Invertebrate Pathology - One Man's Estimate."

August 20. 9:00-12:00 a.m. Special Symposium sponsored by the American Society of Animal Science & the Poultry Science Association entitled "Challenges in Living Systems."
[Marsh Life Science, Benedict Auditorium]

1:30- 4:00 p.m. Special Interest Meetings.
Organizational Meeting of those interested in the Microsporida (organized by Dr. V. Sprague).
[Terrill Home Economics 222]

August 20. 6:00- 8:00 p.m. Society's Happy Hour (No Host).
(Purchase tickets at the Registration Table on a "first come, first serve" basis.
[Holiday Inn]

August 21. 9:00-12:00 a.m. Contributed Papers, Session 4.
[Terrill Home Economics 108]

1:30- 4:00 p.m. Contributed Papers, Session 5.
[Terrill Home Economics 108]

8:00-10:00 p.m. Society's Annual Business Meeting.
[Terrill Home Economics 108]

SCHEDULE

SESSION 1
Monday Afternoon, August 18

Chairman: Dr. John D. Briggs

1:30- 1:45 p.m. Apparent *in vivo* Pathway of a Granulosis Virus Invasion and Infection ..... M. D. Summers
The Cell Research Institute and the Dept. of Botany
University of Texas
Austin, Texas

1:45- 2:00 p.m. Studies on the Alkali-Released Granulosis Virus of *Trichoplusia ni*: Density Gradient Purification and Some *in vitro* Chemical and Physical Properties of the Virus and Virus Components ..... M. D. Summers and J. D. Paschke
The Cell Research Institute and the Dept. of Botany
University of Texas
Austin, Texas

2:00- 2:20 p.m. Replication of an Invertebrate Virus in Vertebrate Cells ..... E. Kurstak, J.-R. Côté, and S. Belloncik
Dept. of Microbiology & Immunology
University of Montreal
Montreal, Canada

2:20- 2:30 p.m. Interaction Between a Granulosis and a Nuclear-Polyhedrosis Virus in the Larva of the Armyworm, Pseudaletia unipuncta ..... Y. Tanada and T. Hukuhara, Div. of Entomology, Univ. of California, Berkeley, California

2:30- 2:45 p.m. Tests on Additives to Prolong Activity of Virus on Foliage ..... R. P. Jaques, Research Station, Canada Department of Agriculture, Harrow, Ontario, Canada

2:45- 3:00 p.m. Recess

3:00- 3:15 p.m. Behaviour of Milky Disease Pathogens in Insect Tissue Culture ..... P. Lüthy*, Ch. Wyss**, and L. Ettlinger ** *Insect Pathology Research Institute, Saulte Ste. Marie and ** Swiss Federal Institute of Technology, Canada

3:15- 3:30 p.m. Development and Fine Structure of a Poxlike Virus Infecting Estigmene acrea Larvae ..... R. R. Granados, D. W. Roberts, and M. Bergoin, Boyce Thompson Institute for Plant Research, Yonkers, New York

3:30- 3:45 p.m. Inclusion Formation and Virus Occlusion in a Nuclear Polyhedrosis and Granulosis of Trichoplusia ni. ... H. J. Arnott and M. D. Summers, The Cell Research Institute, University of Texas, Austin, Texas

3:45- 4:00 p.m. Viral Replication in the Tent Caterpillar (M. disstria) midgut Infected with a Cytoplasmic Polyhedrosis Virus ..... Y. Hayashi and T. Kawarabata, Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada

SESSION 2
Tuesday Morning, August 19

Chairman: Dr. Albert K. Sparks

9:00- 9:15 a.m.	Insecticidal Mycotoxins from *Aspergillus flavus*	R. L. Beard and G. S. Walton The Connecticut Agricultural Experiment Station New Haven, Connecticut
9:15- 9:30 a.m.	A fungus Disease of Plant-Parasitic Nematodes	R. M. Sayre Nematology Investigations, USDA, ARS, CRD Plant Industry Station Beltsville, Maryland
9:30- 9:45 a.m.	The Role of Two Pathogens in Natural Control of *Porthetria dispar*.....	C. C. Doane The Connecticut Agricultural Experiment Station New Haven, Connecticut
9:45-10:00 a.m.	Three Fungi Tested for Control of the Cowpea Curculio, *Chalcodermus aeneus* (Boheman).	J. V. Bell Entomology Research Division USDA, ARS Charleston, South Carolina
10:00-10:15 a. m.	Recess	
10:15-10:30 a.m.	X-irradiation and *Pseudomonas*-Susceptibility of Larvae of *Tenebrio molitor*	H. S. Ducoff, B. Mortimore, and J. L. Crossland Dept. of Physiology and Biophysics University of Illinois Urbana, Illinois
10:30-10:45 a.m.	A Progress Report on a New Coccidian in a Beetle Larva (*Trogoderma parabile*)	L. Tan and H. E. Welch Department of Zoology University of Manitoba Winnipeg, Canada

10:45-11:05 a.m. Comparative Activities of Different Isolates of Bacillus thringiensis var alesti Against the Tobacco Budworm .....

H. T. Dulmage
USDA, ARS, P.O. Box 1033
Brownsville, Texas

11:05-11:25 a.m. Characterization of Cytoplasmic Polyhedrosis Virus RNA .....

Y. Hayashi and T. Kawarabata
Insect Pathology Research Institute
Saulte Ste. Marie
Ontario, Canada

11:25-11:40 a.m. Infection of the Boll Weevil by Chilo Iridescent Virus .....

R. E. McLaughlin and
H. A. Scott
USDA, ARS
Entomology Research Division
Boll Weevil Research Laboratory
State College, Mississippi

11:40-11:50 a.m. Boll Weevil Tissue Culture .....

R. E. McLaughlin, M. R. Bell, and G. B. McReynolds
USDA, ARS
Entomology Research Division
Boll Weevil Research Laboratory
State College, Mississippi

SESSION 3
Tuesday Afternoon, August 19

Chairman: Dr. Charles S. Richards

1:30-1:45 p.m. Basal Cell Hyperplasia in the Soft-Shell Clam, Mya arenaria .....

M. M. Barry and P. P. Yevich
National Marine Water Quality Laboratory
West Kingston, Rhode Island

1:45-2:00 p.m. Pathology of Labyrinthomyxa Disease in Bivalve Molluscs .....

J. G. Mackin
Department of Biology
Texas A & M University
College Station, Texas

2:00-2:15 p.m. Effect of Host Age on the Development of Trematode Larvae .....

J. E. Ubelaker
Department of Biology
Southern Methodist University
Dallas, Texas

2:15-2:35 p.m. An Amoeba Associated with Mass Mortalities of Crassostrea commercialis in Tahiti, French Polynesia .....

T. C. Cheng
Department of Biology
Lehigh University
Bethlehem, Pennsylvania

2:35-2:50 p.m. Recess

2:50-3:00 p.m. Inheritance of Abnormal Tissue Growths in the Pulmonary Cavity of Biomphalaria glabrata .....

C. S. Richards
Laboratory of Parasitic Diseases
National Institutes of Health
USPHS
Bethesda, Maryland

3:00-3:15 p.m. An Epizootic Disease with Sarcomatoid Affinities in the Edible Mussel, Mytilus edulis.....

C. A. Farley
U. S. Dept. of the Interior
Fish & Wildlife Service
Bureau of Commercial Fisheries
Biological Laboratory
Oxford, Maryland

3:15-3:30 p.m. Hemolymph Free Amino Acid Composition of Crassostrea virginica infected with Bucephalus sp. and Minchinia nelsoni .....

S. Y. Feng, E. A. Khairallah, and W. J. Canzonier
Marine Research Laboratory and Biological Sciences Group
University of Connecticut
Noank, Connecticut and
The New Jersey Oyster Research Laboratory
Rutgers, The State University
New Brunswick, New Jersey

3:30-3:45 p.m. Host Cellular Response to Larval Trematode Transplants in Helisoma duryi normale .....

H. W. F. Yee and T. C. Cheng
Department of Biology
Lehigh University
Bethlehem, Pennsylvania

3:45- 4:00 p.m. Preliminary Studies on Tissue Transplant Immunity in Gastropod Molluscs ..... P. C. Galloway and T. C. Cheng, Department of Biology, Lehigh University, Bethlehem, Pennsylvania

SESSION 4
Thursday Morning, August 21

Chairman: Dr. S. Y. Feng

9:00- 9:15 a.m. Lack of a Bactericidal Response in the Earthworm Lumbricus terrestris after Immunization with Bacterial Antigens ..... E. L. Cooper, R. T. Acton, P. Weinheimer, and E.E. Evans, Department of Anatomy, School of Medicine, University of California, Los Angeles, California and Department of Microbiology, University of Alabama, Birmingham, Alabama

9:15- 9:30 a.m. Cytological Abnormalities in Tissue Cultured Cells Treated with Extracts from Sponges..... J. T. Cecil, M. F. Stempien,Jr., and R. F. Nigrelli, Osborn Laboratories of Marine Sciences, Boardwalk & W. 8th St., Brooklyn, New York

9:30- 9:45 a.m. The Behavior of ^{3}H-thymidine Labeled Leucocytes of the Bivalve Mollusc, Tapes semidecussata..... D. P. Cheney and A. K. Sparks, College of Fisheries, University of Washington, Seattle, Washington

9:45-10:00 a.m. A Preliminary Report on the Histopathological Effects of Ionizing Radiation on the Digestive System of Crassostrea gigas and Subsequent Repair ..... M. C. Mix and A. K. Sparks, College of Fisheries, University of Washington, Seattle, Washington

10:00-10:15 a.m.	*Vibrio parahaemolyticus*: Relationships of Strains Isolated from Human Enteritis Outbreaks in Japan and from Diseased Blue Crabs (*Callinectes sapidus*) in Chesapeake Bay	T. E. Lovelace and R. R. Colwell Department of Biology Georgetown University Washington, D. C.
10:15-10:30 a.m.	Recess	
10:30-10:45 a.m.	The Reaction of Surface Burns (Branding) in the Crayfish	E. Johnson and A. K. Sparks College of Fisheries University of Washington Seattle, Washington
10:45-11:00 a.m.	Histochemical and Histopathological Studies on Corals, *Porites* spp., Parasitized by Trematode Metacercariae	A. Wong and T. C. Cheng Department of Biology Lehigh University Bethlehem, Pennsylvania
11:00-11:15 a.m.	Microbial Infection in Regenerating Sea-Urchin Spines: Preliminary Observations	P. T. Johnson Center for Pathobiology University of California Irvine, California
11:15-11:35 a.m.	Microbial Insect Control - An Interdisciplinary Approach	B. Maksymiuk Forestry Sciences Laboratory Corvallis, Oregon
11:35-11:50 a.m.	Comparisons Between Different Pox-Like Virus Diseases in Insects ...	M. Bergoin and C. Vago Station de Recherches Cytopathologiques Saint-Christol Montpellier, France

SESSION 5
Thursday Afternoon, August 21

Chairman: Dr. John C. Harshbarger

1:30-1:45 p.m.	Epizootiological Observations on the Microsporidioses of Heliothis Species and the Larval Parasites Campoletis perdistinctus and Cardiochiles nigriceps in North Carolina	W. M. Brooks Department of Entomology North Carolina State University Raleigh, North Carolina
1:45-1:30 p.m.	The Effect of Gamma Radiation on Growth Development and Mortality of Hemerocampa leucostigma	E. A. Hoffman and H. W. Rossmoore Department of Biology Wayne State University Detroit, Michigan
1:30-1:45 p.m.	The Fungus Metarrhizium anisopliae as a Pathogen of Mosquito Larvae	D. W. Roberts Boyce Thompson Institute for Plant Research Yonkers, New York
1:45-2:00 p.m.	Ultrastructual Observations on a Spirochetal Infection of the Brine Shrimp, Artemia salina	G. E. Tyson Dept. of Biological Structure University of Washington Seattle, Washington
2:00-2:15 p.m.	The Advantages of Pathological and Oncological Research with Invertebrates	H. E. Kaiser Department of Anatomy School of Medicine George Washington University Washington, D. C.
2:15-2:30 p.m.	Incomparabilities of Specific Characteristic Plant and Animal Tissues	H. E. Kaiser Department of Anatomy School of Medicine George Washington University Washington, D. C.

2:30-2:45 p.m. Mitoses in Thin Sections of Asexual Hydras ..... Carlton H. Nadolney
Beekman-Downtown Hospital
170 William Street
New York, New York

2:45-3:00 p.m. The Fine Structure of Some of the Developmental Stages of Mattesia grandis McLaughlin (Sporozoa, Neogregarinida) parasite of the Boll Weevil Anthonomus grandis Boheman ..... Jiri Vavra
College of Veterinary Medicine
University of Illinois
Urbana, Illinois, and
Roy E. McLaughlin
U. S. Dept. of Agriculture
Boll Weevil Research Laboratory
State College, Mississippi

ABSTRACTS OF PAPERS

SESSION 1
Monday Afternoon

Apparent *in vivo* Pathway of a Granulosis Virus Invasion and Infection. M. D. Summers, The Cell Research Institute and the Department of Botany, The University of Texas, Austin, Texas.

Early stages of granulosis virus entry and infection of host cells were studied by electron microscopy in *Trichoplusia ni* larvae. Preliminary studies indicate that unique mechanisms of virus entry and release of the virus genome may exist.

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Studies on the Alkali-Released Granulosis Virus of *Trichoplusia ni*: Density Gradient Purification of some *in vitro* Chemical and Physical Properties of the Virus and Virus Components. M. D. Summers and J. D. Paschke, The Cell Research Institute and the Department of Botany, The University of Texas, Austin, Texas.

Trichoplusia ni granulosis virus and alkali-liberated virus particles were purified by a combination of differential and sucrose density-gradient ultracentrifugations. Virus and virus components separated by this method were identified by electron microscopy. Some of their chemical and physical properties were determined in the absence of the inclusion body.

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Replication of an Invertebrate Virus in Vertebrate Cells. E. Kurstak, J.-R. Côté, and S. Belloncik, Department of Microbiology & Immunology, University of Montreal, Montreal, Canada.

Densonucleosis virus (DNV) is a DNA, naked icosahedral, 200 Å virus showing 42 capsomeres. DNA: protein ratio of 37:63. It is very virulent and of polytropic nature for its original host *Galleria mellonella* larvae. Cytopathic effects are observed in mice L cells 4 days after inoculation with DNV. They show a roundness in their shape followed by clustering and many syncytium are present. Feulgen positive intra-nuclear inclusions may be observed. Antigenic material of DNV in the cytoplasm of L cells is present 24 hours after infection. Later heavy intranuclear fluorescent is seen. The replication of DNV virions in L cells is observed. Infection of healthy larvae is done with those virions. This first case of replication of an invertebrate virus in vertebrate cells, proved the present interest in comparative virology.

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Interaction Between a Granulosis and a Nuclear-Polyhedrosis Virus in the Larva of the Armyworm, *Pseudaletia unipuncta*. Y. Tanada and T. Hukuhara, Division of Entomology, University of California, Berkeley, California.

A granulosis virus of the armyworm, *Pseudaletia unipuncta*, increases the incidence of infection by a nuclear-polyhedrosis virus when the two viruses are fed together to the armyworm. The increase in incidence has been analyzed

quantitatively. Gnotobiotic study with the use of these two viruses and the armyworm, indicates that other microorganisms do not play a significant role in the interaction of the two viruses. The granulosis virus has been separated into major components, and their role in the interaction has been investigated.

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Tests on Additives to Prolong Activity of Virus on Foliage. R. P. Jaques, Research Station, Canada Department of Agriculture, Harrow, Ontario, Canada.

The activity of the nuclear-polyhedrosis virus of Trichoplusia ni is reduced by more than 75% in 4 days after application to leaves of plants in plots. Some proteinaceous materials, including soy hydrolysate and peptonized milk, reduce the rate of inactivation of the virus, considerably prolonging activity of the residues exposed to sunlight or an artificial source of ultraviolet light.

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Behaviour of Milky Disease Pathogens in Insect Tissue Culture. P. Luthy,* Ch. Wyss,** and L. Ettlinger.** *Insect Pathology Research Institute, Sault Ste. Marie, ** Swiss Federal Institute of Technology, Canada.

An in vitro system to study vegetative growth and sporulation of pathogens causing milky disease was developed. The organisms used in the experiments were two European isolates from Melolontha melolontha and an American strain of B. popilliae Dutky from Popillia japonica.

Spores or vegetative cells were inoculated in 30 ml tissue culture flasks containing 5 ml of Grace's medium and 0.2 ml of larval hemolymph of a species of Phyllophaga (Scarabeidae, Coleoptera). Spores of all three strains germinated readily in this environment and good vegetative growth was obtained. The European isolates gave an excellent sporulation rate, while B. popilliae Dutky grew abundantly but did not sporulate at all.

As soon as the spores were mature they disappeared out of the medium and were found to be phagocytised by the hemocytes. There was no evidence that the blood cells were also able to phagocytise vegetative cells. A parallel in vivo study with Melolontha melolontha as host revealed similar phagocytic action of hemocytes towards the three milky disease strains.

The usefulness of the above mentioned in vitro system is discussed in connection with the taxonomy and the mode of action, as well as with the successful cultivation of milky disease pathogens in the presence of host hemolymph.

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Development and Fine Structure of a Poxlike Virus Infecting Estigmene acrea Larvae. Robert R. Granados, Donald W. Roberts, and Max Bergoin, Boyce Thompson Institute for Plant Research, Yonkers, New York.

A virus which is morphologically similar to the poxviruses of animals and man was found to multiply in Estigmene acrea (Lepidoptera: Arctiidae) larvae.

Immature forms of the virus were formed in viroplasms in the cytoplasm of adipose cells and hemocytes. Viroplasms were generally found near the nucleus and were composed of an electron dense, granular material. Immature particles measured approximately 3500 Å in diameter and were surrounded by two unit membranes. Mature virions were found either free in the cell cytoplasm or occluded within spheroidal, protein inclusion bodies. Mature virions were oval-shaped and measured 2500 Å X 3500 Å in size. The virions possessed an outer coat composed of spherical units approximately 300 Å in diameter. Virus particles had a core or nucleoid surrounded by a lateral body. The coat of the core was composed of two layers; an outer layer 110 Å wide and an inner one 75 Å wide. The inner layer was smooth but the outer layer was composed of subunits 110 Å in length and 60 Å in diameter. Rod-like structures 300 Å in diameter and of unknown length were observed in the viral core. Bundles of cytoplasmic fibrils were common in infected cells. The fibrils were 80 Å in diameter and their length sometimes extended through the entire cell.

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Inclusion Formation and Virus Occlusion in a Nuclear Polyhedrosis and Granulosis of Trichoplusia ni. Howard J. Arnott and Max D. Summers, The Cell Research Institute, The University of Texas, Austin, Texas.

The formation of viral and non-viral inclusions in nuclear polyhedrosis virus infected cells of Trichoplusia ni is described. A relationship between "membrane-like" profiles, fibrous material, and inclusion bodies was shown which is believed to reveal a possible process by which protein sub-units are assembled into a growing crystal. During the development fibers were first observed forming between existing "membrane-like" profiles. The origin of the profiles is unknown, but they are of a composite structure not previously shown in biological systems. Observations suggest that the profiles are related to or intimately associated with tubules having a unit membrane structure; they may possibly be derived from the nuclear membrane. The fibrous material associated with profiles also appears to be fused with the surface of growing crystals. A partly ordered structure exhibiting a line periodicity similar to that of crystals can be seen in the fibrous material.

A similar fibrous material was also observed in granulosis virus infected fat body cells and was often intimately associated with growing, but aberrant, inclusion formation. However, "membrane-like" profiles were not observed in the replication of the granulosis virus.

Inclusions may be formed in the presence of large numbers of naked virus particles, but viral occlusion only occurs after acquisition of the outer membrane. Possible explanations for these observations are discussed.

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Viral Replication in the Tent Caterpillar (M. disstria) Midgut Infected with a Cytoplasmic Polyhedrosis Virus. Y. Hayashi and T. Kawarabata, Insect Pathology Research Institute, Saulte Ste. Marie, Ontario, Canada.

The synthesis of cellular RNA continues in the midguts of larvae infected with a cytoplasmic polyhedrosis virus. It is rapidly blocked (90%) by actinomycin D at a concentration of 1 µg/larva. At this concentration, actinomycin D does not inhibit the synthesis of viral RNA. Three species of virus-induced RNA

can be obtained from infected cell. A heavier RNA (22S) presumably single stranded, is sensitive to ribonuclease and the other two (15 and 12S) are partially resistant to the enzyme. These enzyme resistant portions of these latter two RNA's are double-stranded and are released from the virions in the cell, while the sensitive parts are single stranded suggesting the viral specific m-RNA. In fact, virus induced RNA was extracted from the polyribosomal fraction as single stranded in the presence of actinomycin D.

On the other hand, the assembling of viral RNA and protein to form virions was carried out in the presence of antibiotics. No inhibition of virion formation occurs by actinomycin D (1-2 μg/larva), but 100% inhibition by puromycin (50 μg/larva). The mechanism of viral replication will be discussed.

SESSION 2
Tuesday Morning

Insecticidal Mycotoxins from Aspergillus flavus. R. L. Beard and G. S. Walton, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven, Connecticut.

Water extracts of cultures of Aspergillus flavus var. columnaris have insecticidal properties against young stages of several species of insects. Kojic acid has been identified as one such product, but others result from different media in which the fungus is grown. Studies on the production, isolation, and characteristics of these metabolic products and their effects on insects will be reported.

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A Fungus Disease of Plant-Parasitic Nematodes. Richard M. Sayre, Nematology Investigations, U.S.D.A., ARS, CRD, Plant Industry Station, Beltsville, Maryland.

A phycomycetous fungus, Catenaria anguillulae, a parasite of phytonematodes, was isolated from greenhouse soil and cultured. Zoospores of the fungus were tested for their virulence to the nematodes, Panagrellus redivivus and Ditylenchus dipsaci. A linear relationship was shown to exist between increasing numbers of zoospores and the incidence of the fungus disease in nematodes in vitro. An approximation of the number of zoospores necessary to cause 50% mortality of the two nematode species was made. The fungus developed more rapidly at 28°C than at the lower temperatures. D. dipsaci was less susceptible to attack from the fungus than P. redivivus. A pH of 9 and a low salt concentration favored the rapid development of the fungus in nematodes. Many more zoospores were required in sand and soil to achieve the same incidence of the disease than in liquid cultures. The tests indicated that this fungus isolate was not a very effective biological control agent under conditions used.

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The Role of Two Pathogens in Natural Control of Porthetria dispar. Charles C. Doane, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, Connecticut.

The nuclear polyhedrosis virus (NPHV) and a variant of Stretococcus faecalis are apparent, primary pathogens of the gypsy moth. NPHV is passed from generation-to-generation on the surfaces of eggs. There is high frequency of transmission and subsequent latent infection. Susceptibility of first-instar larvae may vary greatly and is due, in part, to environmental influences on the unhatched larva. The mortality of first-instar larvae from different field populations ranged from less than 5% to over 80%. These laboratory studies of individually reared larvae showed that mortality from infection from the egg was most likely to occur in the first instar. Those surviving the first instar rarely died from polyhedrosis.

Field studies on the development of epizootics indicate that, after the first instar, disease spread is density-dependent and that the behavior of the larvae increases relative density. S. faecalis was an important pathogen in the outbreaks and its pathogenic and cultural characteristics will be discussed as time permits. It is found commonly in disease outbreaks in the field in association with NPHV. It was highly pathogenic to laboratory-reared, virus-free larvae and produced symptons similar to those observed in the field.

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Three Fungi Tested for Control of the Cowpea Curculio Chalcodermus aeneus (Boheman). James V. Bell, U.S.D.A., ARS, Entomology Research Division, Box 3-187, Charleston, South Carolina.

Larvae of the cowpea curculio Chalcodermus aeneus (Boheman) were laboratory tested for susceptibility to fungus diseases. Spicaria rileyi was unable to infect the insect, but two other closely related fungi, Metarrhizium anisopliae and Beauveria bassiana, each significantly reduced curculio populations when the conidia were applied directly from sporulating laboratory cultures or after they were air-dried in soil for 40 days. Beauveria was also pathogenic to adults. In small field tests only Metarrhizium was effective as a control agent.

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X-Irradiation and Pseudomonas-Susceptibility of Larvae of Tenebrio molitor. H. S. Ducoff, Betty Mortimore, and Janice L. Crossland, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois.

Larvae of Tenebrio molitor are highly susceptible to injected Pseudomonas aeruginosa. Injection of 200 to 300 bacteria (in 3-6 ul of Grace's medium) killed at least 90% of the larvae within 4 days; injection of 20-30 bacteria killed 50% or less of the larvae within 1 week, with few additional deaths in the following 2 weeks. Larvae exposed to X-ray doses of 4000 R or more rarely develop into viable adults, but usually survive to pupation, and even after doses of 15000 R, larvae survive for many weeks. X-ray doses of 5000 R had no detectable effect on the response of larval Tenebrio to subsequent injections of Pseudomonas, but when doses of 9000 or 12000 R were administered

24 hours prior to injection of 20-30 pseudomonads, most larvae died within 4 days. We have reported earlier that endogenous infection does not contribute to the acute lethal syndrome in irradiated adults of Tribolium; the present experiments indicate that mid-lethal X-ray doses do not affect susceptibility to injected pathogens, but supra-lethal doses do interfere with the disease-resistance processes of larvae of Tenebrio. (Work supported by Grant HD 3163, U. S. Public Health Service).

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Comparative Activities of Different Isolates of Bacillus thuringiensis var alesti against the Tobacco Budworm. Howard T. Dulmage, U.S.D.A., ARS, P. O. Box 1033, Brownville, Texas.

While it was known for some time that the production of insecticidal activity by isolates of Bacillus thuringiensis varies according to the serotype tested, it is of interest to know if this variation also exists between different isolates of the same serotype. This paper reports a survey of the insecticidal activity of several isolates of B. thuringiensis var alesti as determined using the tobacco budworm, H. virescens, as a test organism. Results are reported in International Units as determined through comparative bioassays with the proposed standard E-61. The significance of this data for the development of this microbiological insect control agent is discussed.

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Characterization of Cytoplasmic Polyhedrosis Virus RNA. Y. Hayashi and T. Kawarabata, Insect Pathology Research Institute, Saulte Ste. Marie, Ontario, Canada.

Viral RNA was extracted by phenol method from purified virions. This RNA appears as fibrous form in alcohol, ether and acetone similar to that of DNA. The molar ratio of base compositions is complementary i.e., ratios of adenine/uracil and guanine/cytosine are nearly the same. The guanine/cytosine content is about 43% of total nucleotide. Two species of RNA, with sedimentation constants of 12 and 15S were extracted from the virions. On electrophoresis in polyacrylamide gels these size classes can be further resolved to at least 5 fragments of RNA. These fragments are resistant to ribonuclease provided that the concentrations of ionic strength, as well as that of the enzyme, are suitably adjusted. They exhibit a sharp melting profile with a Tm.

By these criteria, the viral RNA was considered as double stranded.

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Infection of the Boll Weevil by Chilo Iridescent Virus. R. E. McLaughlin and H. A. Scott, U.S.D.A., ARS, Entomology Research Division, Boll Weevil Research Laboratory, P. O. Box 5367, State College, Mississippi.

Chilo iridescent virus, injected intrahemocoelically or ingested, infected larval and adult boll weevils (Anthonomus grandis). Virus from infected weevils infected the wax moth, Galleria mellonella, and was also serologically identical

to the initial material. Probit-analysis of mortality-response-data to varied concentrations of a standard suspension fed to adult weevils showed a definite correlation to virus concentration. The virus, incorporated in a viscous feeding-stimulant bait placed on a rooftop or sprayed on cotton foliage in field cages infected weevils up to 7 days, and even at 14 days continuous exposure some infective virus remained. Protection of a non-inclusion type virus by formulations such as those used as baits for the boll weevil encourage further investigations for possible control usage.

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Boll Weevil Tissue Culture. R. E. McLaughlin, M. R. Bell, and G. B. McReynolds, USDA, ARS, Entomology Research Division, Boll Weevil Research Laboratory, P. O. Box 5367, State College, Mississippi.

Embryonic tissue from boll weevils (Anthonomus grandis) was used in attempts to develop primary cell cultures from which cell lines could be grown, and which might also enable us to culture ovarian tissue. Embryonic tissue has been maintained in primary cultures, with cell movement highly active as long as 5 days. Cells from early eyespot stage embryos were grown for 36 days in cultures and produced large cell aggregates, some of which were 730 microns in diameter. They were unattached to the glass, and formed rounded, apparently hollow structures. These tissues were pulsing at 30 days.

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SESSION 3
Tuesday Afternoon

Basal Cell Hyperplasia in the Soft-Shell Clam, Mya arenaria. Marcia M. Barry and Paul P. Yevich, National Marine Water Quality Laboratory, P. O. Box 277, West Kingston, Rhode Island.

Preliminary examination of soft-shell clams, Mya arenaria, from one sampling point in Point Judith Pond, Narragansett, Rhode Island showed a number of animals with focal areas of marked basal cell hyperplasia of gill filaments and extensive hyperplasia of kidney epithelium which involves the entire kidney. The study will be expanded to include samples collected from other sites in Pt. Judith Pond as well as from other bays and estuaries along the Rhode Island coast.

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Pathology of Labyrinthomyxa Disease in Bivalve Mollusca. J. G. Mackin, Texas A&M University, Department of Biology, College Station, Texas.

Certain diseases of bivalve molluscs such as the Australian Winter Disease, Denman Island disease, Malpeque Bay disease, and several others caused by Labyrinthomyxa spp, have a common pathological syndrome, involving primary attack on gill, mantle, and gut epithelia, bacterial complication and bacteremia, and invasion and lysis of Leidig cell tissue. A new method of diagnosis of

these diseases is presented. Small populations of oysters are rid of bacteria by menas of antibiotic treatment, and subjected to stress. Mantle from oysters in advanced disease is used to make impression mounts on slides and the parasites thus transferred are stained variously and diagnosis made under the microscope.

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Effect of Host Age on the Development of Trematode Larvae. John E. Ubelaker, Department of Biology, Southern Methodist University, Dallas, Texas.

Age-related differences in pelecypods are presented relative to their susceptibility to trematode infections. The data presented indicate that Pisidium adamsi can be infected at any age and that the length of time required for development of the larvae to the cercarial stage is dependent upon age of the pelecypod. Mortality of clams is probably related to mechanical damage or hypoglycemia.

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An Amoeba Associated with Mass Mortalities of Crassostrea commercialis in Tahiti, French Polynesia. Thomas C. Cheng, Dept. of Biology, Lehigh Univ., Bethlehem, Pennsylvania.

Attempts to establish a commercial oyster industry at Tarávao, Tahiti, French Polynesia, during the last five years have met with little success due to mass mortalities. Specimens of Crassostrea commercialis collected from this site were flown to Hawaii for examination. Although the valves of these oysters were tightly held together by the adductor muscles upon arrival, histological examinations revealed that the various body tissues were undergoing necrotic alterations comparable to those described by Sparks and Pauley (1964). Numerous trophic amoebae, averaging 0.010 mm in greatest diameter, were found in the lumina of the stomachs and intestines. In addition, amoebae were found in the process of penetrating the lining epithelium, in the interacinar spaces and penetrating the acini of the digestive gland, intermingled with Leydig cells, and in the gonads. These amoebae are characterized by the presence of a vesicular nucleus, production of lobopodia, and conspicuous food vacuoles. A nonspecific leucocytosis was observed. It is not known whether these amoebae are the causative agents of the mortalities or secondary invaders.

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Inheritance of Abnormal Tissue Growths in the Pulmonary Cavity of Biomphalaria glabrata. Charles S. Richards, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland.

In the course of studies on biological control of Biomphalaria glabrata, molluscan intermediate host and vector of Schistosoma mansoni, an abnormal growth was occasionally observed in the pulmonary cavity. Examination of fresh tissues and fixed and stained sections has so far failed to incriminate an etiologic agent.

Genetic records enabled tracing the occurrence of the growths back to a common source, suggesting inheritance was involved. Selection, isolated rearing, and self-fertilization resulted in strains with increased frequency, and cross-fertilization demonstrated transmission of the tendency for production of the growths. Evident in snails only a few days old, the growths maintained consistent size and location in individual snails, growing proportionately with the rest of the snail.

Small growths had no apparent effect on the snails, but extensive growths occluded the pulmonary cavity and made retraction into the shell impossible. Information on inheritance of abnormal growths in B. glabrata, as a genetic model, may help explain occurrence of some growths in other mollusks.

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An Epizootic Disease with Sarcomatoid Affinities in the Edible Mussel, Mytilus edulis. C. Austin Farley, Fish & Wildlife Service, Biological Laboratory, Bureau of Commercial Fisheries, U. S. Department of the Interior, Oxford, Maryland.

In two samples of live mussels (Mytilus edulis) from Yaquina Bay, Oregon, 3 of 43 (7%) received on September 4, 1968, and 7 of 57 (12%) received on February 4, 1969, had proliferative invasive lesions which consisted of undifferentiated hemocyte-like cells. These cells were characterized by ovoid or indented interphase nuclei, 7 to 13μ in diameter (2 to 4 times larger than normal hemocyte nuclei). Nuclei contained a reticulated pattern of deoxyribonucleic acid (DNA) and 1 to as many as 5 DNA-ringed feulgen-negative nucleoli. Mitotic figures were common which showed the same degree of enlargement as interphase nuclei. This feature appeared to be due to excessive numbers of chromosomes. Tripolar figures and displaced chromosomes were interpreted as evidence for polyploidy. Live cells were agranular, amoeboid, and very sluggish; their movement resembled most closely that seen in hyaline hemocytes. Binucleate cells were reportable and were interpreted as evidence for asynchronous division (karyokinesis without concommitant cytokinesis).

Early cases, in which lesions were localized in the vesicular connective tissue of the mantle, showed no gross pathology. Advanced cases had dense disseminations of atypical cells throughout the tissues, with signs of degenerative changes (pyknosis and reduced mitotic activity in "neoplastic" cells) and marked suppression of gametic development. Mussels in this phase of the disease usually showed gross emaciation and weakness which, combined with observed cytologic degenerative changes, implies a terminal phase of the disease.

The origin of these atypical proliferative cells is unknown. Their morphologic characteristics, the occurrence of early foci in the connective tissue, and the diffuse nature of the invasion, however, suggest a reticular (hemolymph system) origin. The disorder has many features similar to reticulum cells sarcoma or leukemia of vertebrates.

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Hemolymph Free Amino Acid Composition of Crassostrea virginica Infected with Bucephalus sp. and Minchinia nelsoni. S. Y. Feng, E. A. Khairallah and W. J. Canzonier, Marine Research Laboratory and Biological Sciences Group, University of Connecticut, Noank, Connecticut and New Jersey Oyster Research Laboratory, Rutgers, The State University, New Brunswick, New Jersey.

Oysters obtained from the Navesink River, New Jersey, were exposed to the infection of Minchinia nelsoni by transplanting them to Delaware Bay, an endemic area of the disease. In June 1967, after a 9-month residence in the Bay, the animals were exsanguinated and fixed for hemolymph free amino acid as well as histopathologic analyses. Based on histopathologic examinations, oysters were divided into four groups: the normals (12), the Bucephalus-infected (9), the M. nelsoni-infected (13) and the Bucephalus-Minchinia-infected (3). Hemolymph samples were pooled according to the four categories. The pooled samples were deproteinized and ultrafiltered; the ultrafiltrates were applied to the columns of Beckman Model 120C Amino Acid Analyzer.

The results showed distinct qualitative and quantitative changes in the composition of hemolymph free amino acids of the infected oysters, as contrasted with the normals. Twelve amino acids were common to all groups, although concentrations of these amino acids were often quite variable in infected oysters. Arginine and lysine were detected only in the normal and Bucephalus-infected groups respectively. The presence of γ-aminobutyric acid was an unique feature of the hemolymph of the Minchinia-infected and Bucephalus-Minchinia-infected oysters, while phosphoserine was found exclusively in the normal and Bucephalus-infected oysters. Taurine, which was present in high concentrations, was the only sulphur-containing compound detected. The concentrations of taurine and phosphoethanolamine in all infected oysters were reduced to approximately one half of the normals. Glutamic acid, glycine, alanine and B-alanine were present in relatively high concentrations and exhibited considerable quantitative fluctuations in the infected oysters. No free aromatic amino acids were detected in the normal and infected oysters. The possible metabolic significance of the data will be discussed.

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Host Cellular Response to Larval Trematode Transplants in Helisoma duryi Normale. Herbert W. F. Yee and Thomas C. Cheng, Department of Biology, Lehigh University, Bethlehem, Pennsylvania.

Third generation rediae of the avian fluke, Philophthalmus gralli, were transplanted into the hepatopancreas and cephalopedal sinus regions of the snail, Helisoma duryi normale. The rediae were obtained from infected regions of Tarebia granifera, washed in BSS or physiological saline, concentrated by centrifugation, and injected into the host snails with a hypodermic syringe fitted with glass needle. Examination of paraffin mounted, haematoxylin and eosin stained sections of snail tissue fixed at specific time intervals showed moderate host reaction up to 24 hour following transplantation. There was amoebocytic infiltration into the rediae found within the cephalopedal sinus with no evidence of fibroblastic involvement. At 48 hours, the connective tissue regions around the cercariae became more compact. A hundred and twenty

hours after transplantation, the cercariae were isolated by hypertrophic transformed fibroblasts. Granuloma development was quite pronounced at 240 hours with extensive fibroblastic accumulation around a central core of intact or degenerating cercaria infiltrated with amoebocytes.

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Preliminary Studies on Tissue Transplant Immunity in Gastropod Molluscs. Peter C. Galloway and Thomas C. Cheng, Department of Biology, Lehigh University, and Department of Zoology, University of Hawaii.

Digestive gland tissues from Helisoma duryi normale (HI-3) (allografts), H. trivolvis (xenografts), Tarebia granifera (xenografts), and Melania newcombi (xenografts) were transplanted into the cephalopedal sinuses of H. duryi normale (HI-2) and their fates were traced by examining histological sections fixed at 24, 48, 96, 192, and 384 hours post-transplantation. It is apparent that the recipients are capable of differentiating between allografts and xenografts since host reactions directed at the latter are more rapid and generally more severe. The rapid dissociation of the acinar cells of transplanted H. trivolvis and T. granifera suggests that some yet undetermined factor of host origin is responsible for the lysis of these xenogeneic tissues within 24 hours. Cellular capsules surrounding the dissociated cells of H. trivolvis and T. granifera transplants are in the form of hypertrophic fibroblasts and epithelioid cells. Evidences indicate that the latter have differentiated from the former which, in turn, have differentiated from wandering leukocytes. Both H. trivolvis and T. granifera cells are encapsulated by the 48th hour. Eventually, the cellular remnants of these transplants are removed by leukocytic phagocytosis.

In the case of M. newcombi xenografts, the primary type of host reaction is in the form of myofibrous encapsulation which is initiated by the 24th hour. Multinucleate macrophages are also associated with these xenografts at the same time. The transplants, however, are not dissociated during the span of the experiment.

In the case of H. duryi normale allografts, epithelioid cell encapsulation occurs by the 192nd hour but the acini are not dissociated by the 384th hour.

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SESSION 4
Thursday Morning

Lack of Bactericidal Response in the Earthworm Lumbricus terrestris after Immunization with Bacterial Antigens. Edwin L. Cooper, R. T. Acton, P. Weinheimer, and E. E. Evans, Department of Anatomy, School of Medicine, University of California, Los Angeles, California.

To determine whether the earthworm, Lumbricus terrestris, could show a bactericidal response, 775 worms were challenged with various bacterial antigens. They were injected with L1-L6, bacterial strains isolated from the slime and gut cavity of earthworms, EMB-1 from a representative crustacean and Salmonella typhosa H antigen. Controls consisted of worms injected with 0.85% saline,

formalized saline at 0.3%, and shams which were only punctured with a needle without injection of any antigen. Responses were looked for after 12 hours and 7 days at antigenic concentrations of 10^6 and 10^8. In addition, L-1 was injected at concentrations of 10^4 and 10^6. Worms challenged initially with the highest doses were injected again for secondary responses and tested 33-42 days later. Bactericidal responses, assayed by means of the Schwab and Reeves technique, were not observed in any of the experimental groups. Although the earthworm is capable of a specific, apparently, cellular response to allogeneic and xenogeneic tissue antigens, they seem to be incapable of synthesizing detectable humoral substances to certain bacterial antigens. (Supported by research grants E-492 from the American Cancer Society, GB-7607 from the National Science Foundation, AI-02693 from the U.S.P.H.S. and a U.S.P.H.S. training grant AI-00293.)

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Cytological Abnormalities in Tissue Cultured Cells Treated with Extracts from Sponges[1]. Jack T. Cecil, Martin F. Stempien, Jr., and Ross F. Nigrelli, Osborn Laboratories of Marine Sciences, Boardwalk and West 8th Street, Brooklyn, New York.

Aqueous extracts from dried West Indian sponges Verongia archeri, Agelas sp., Homaxinella rudis, Cribrochalina infundabulum, Adocia carbonaria, and Xestospongia muta contain biologically active compounds which induce cytological abnormalities in the KB cell line (human oral carcinoma) and the fish cell lines FHM (fat head minnow), and RTG-2 (rainbow trout gonadal).

Cells were either passed and grown in dilutions containing 25-50 mcg/ml of these sponge extracts, or the cells grown first as monolayers and the sponge extracts applied in similar dilutions in the maintenance medium. Observations were made by fluorescent microscopy of acridine orange stained materials or by light microscopy of hematoxylin-eosin stained preparations. The abnormalities include nuclear fragmentation, formation of micronuclei, nuclear budding, occasional multinucleation and giant cell formation, cell elongation, and formation of cytoplasmic bridges.

Since the extract of Agelas sp. has been identified as an indole derivative, tryptophan and 5 hydroxytryptophan were also tested. Nuclear budding and occasional multinucleation were also observed, but in ten fold higher concentrations than the sponge extracts.

[1]This investigation was supported by a grant from the Scaife Foundation to the Osborn Laboratories of Marine Sciences.

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The Behavior of ^{3}H-thymidine Labeled Leucocytes of the Bivalve Mollusc, Tapes semidecussata. Daniel P. Cheney and Albert K. Sparks, College of Fisheries, University of Washington, Seattle, Washington.

The manila clam, T. semidecussata, was employed as the experimental species to determine DNA labeling parameters, locate sites of leucocyte hematopoiesis and to differentiate cell lines from sequential histological data. One µc/g

of ^{3}H-thymidine (specific act. 3.0 C/mM) in an isotonic carrier was injected directly into the body wall. In both chase and rapid-kill experiments, DNA labeling proceeded rapidly at active cell sites with minimal labeling beginning at 5 minutes post-injection with 15 minutes post-injection activity levels reaching values approaching the standard 60 to 120 minute levels. Whole body relative DNA activity levels (cpm/mg DNA) showed a similar pattern. CT and epithelial cell labeling as percentage of cells labeled exhibited wide variations between tissues and between animals; however, there was a depression of DNA labeling in these tissues correlated with the tidal pattern at the time of sampling with lowest degree of labeling at low tide.

Circulating leucocytes were mainly small and medium hyaline forms (3 to 8μ) with an average cell count of 1650 ± 180 SD per mm^{3}. These cells, large hyaline forms (7 to 14μ) and granulocytes were abundant in the loose CT around sites of active absorption and in areas subject to wound stress. Well discriminated hematopoietic sites were not observed and leucocyte replication became focal only when associated with an inflammatory response. Differential analyses of ^{3}H-TdR labeled leucocytes at day 0 post-injection showed 20% small, 50% medium, and 30% large leucocytes. Nearly all cells had euchromatic nuclei. At day 30 the counts were 40% small, 55% medium, and 5% large, and 50% of the labeled nuclei were heterochromatic. Some labeled granulocytes were also noted at day 30.

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A Preliminary Report on the Histopathological Effects of Ionizing Radiation on the Digestive System of Crassostrea gigas and Subsequent Repair. Michael C. Mix and Albert K. Sparks, College of Fisheries, University of Washington, Seattle, Washington.

Oysters were subjected to whole-body gamma irradiation using the following doses: 1, 1,000, 5,000, 10,000, 20,000, 50,000, 100,000, 200,000 and 400,000 rads. Mortalities appeared four days after irradiation in the 200 and 400 Krad groups and, with one exception, all oysters in these two groups were dead by 11 days. No significant mortalities were observed in any other dosage group.

Three different radiation syndroms in respect to histopathological changes in digestive tissues are described. They are: a subacute syndrome observed in oysters irradiated with 200 and 400 Krads, an acute syndrome observed in the 100, 50 and 25 Krad dosage groups and a transitory syndrome in oysters irradiated with 1, 5 and 10 Krads.

Possible mechanisms of repair, although the conclusions are tentative, are also discussed.

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Vibrio parahaemolyticus: Relationship of Strains Isolated from Human Enteritis Outbreaks in Japan and from Diseased Blue Crabs (Callinectes sapidus) in Chesapeake Bay. T. E. Lovelace and R. R. Colwell, Department of Biology, Georgetown University, Washington, D. C.

Strains of bacteria identified as Vibrio parahaemolyticus were isolated from lethargic and moribund crabs being retained in commercial tanks during "shedding" of soft crabs. The animals were abnormally weak and examination

of their hemolymph showed the presence of large numbers of bacteria. From computer analysis of phenetic data, overall DNA base composition and serological analysis, the crab isolates were identified as V. parahaemolyticus. This microorganism has been isolated from "Shirasu" food poisoning outbreaks in Japan and from tissue lesions of victims believed to have contracted infection while swimming in coastal waters of the United States. Seventy-one Japanese cultures of V. parahaemolyticus (received from Drs. H. Zen-Yoji and R. Sakazaki) and a set of tissue lesion isolates (received from Dr. R. Twedt, U. S. Public Health Service) were compared with the Blue crab isolates. On the basis of computer analysis, overall DNA base composition and serology, all strains of V. parahaemolyticus, regardless of source, were found to be closely related (≥ 75% similarity; 44-46 moles % G + C; common K antigens). Since V. parahaemolyticus has been isolated from diseased Blue crabs and is, basically a marine microorganism, i.e., salt-requiring, it appears that V. parahaemolyticus may be endemic in the estuarine environment, primarily a pathogen of invertebrates and secondarily a human pathogen.

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The Reaction to Surface Burns (Branding) in the Crayfish. Eugene Johnson and Albert K. Sparks, College of Fisheries, University of Washington, Seattle, Washington.

A thermal hot brand was applied on the dorsal surface of the carapace of 36 crayfish, Pacifastacus leniusculus (Stimpson). The animals were periodically sampled from .5 hour through 240 hours post injury and examined histologically to observe wound repair. Wound repair began at .5 hours with the formation of a clot under the burn, necrosis of the chitin secreting epidermis and underlying muscle layer, and some leucocytic infiltration. There was increasing leucocytic infiltration into the area beneath the clot up to 96 hours post injury. Cell differentiation began at 120 hours post injury with the formation of a band of cells located on the ventral surface of the clot. Five months post injury showed formation of protochitin in the wound area with appearance of a muscle and epidermal layer. An abnormal number of leucocytes still existed six months post injury.

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Histochemical and Histopathological Studies on Corals, Porites spp., Parasitized by Trematode Metacercariae. Alan Wong and Thomas C. Cheng, Department of Biology, Lehigh University, Bethlehem, Pennsylvania.

The presence of a hitherto unknown trematode metacercaria is reported from two species of reef-forming corals, Porites compressa and P. lobata, from Hawaii. As a consequence of the presence of this parasite encysted within the gastrovascular cavities of tentacles of polyps, the cells comprising the surrounding epidermis and gastrodermis are greatly compressed and reveal cytopathologic changes. Furthermore, cellular reaction in the form of an incomplete, syncytial wall of host cells surrounds the inner, parasite-secreted, noncellular cyst wall. Both the surrounding host cells and the true cyst wall are periodic acid-Schiff-positive and diastase resistant. When stained with toluidine blue, the cyst wall is alpha metachromatic while the surrounding host cells are beta metachromatic which may be due to polycarboxylates and

polysulfates. In sections stained with alcian blue at pH 0.5, 2.6, and 6.7, the cyst wall and the surrounding cells are positive at pH 2.6 thus indicating the presence of carboxyl groups at these sites. As the result of these and other histochemical tests, a partial characterization of the metacercarial cyst and host tissues has been acertained. Quantitative determinations of the ionic calcium concentrations and measurements of the $CaCO_3$ spicules in parasitized and nonparasitized polyps have revealed some apparently parasite-induced differences.

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Microbial Infection in Regenerating Sea-Urchin Spines. Preliminary Observations. Phyllis T. Johnson, Center for Pathobiology, University of California, Irvine, California.

The spines, test, and certain coelomic cells of Strongylocentrotus and other sea urchins contain naphthaquinone pigments called "echinochromes" that some workers believe may act as algistatic agents. In a regenerating spine echinochrome is deposited at the point of active laying-down of calcite crystals. This area contains disorganized tissues and fragments of the cells involved in providing the calcite, and hence is rich in organic material. In pale urchins, which lack the normal amount of echinochrome, the area of active spine regeneration may be covered superficially with algae and be infected internally with a variety of protistens including diatoms, ciliates, probable fungi, and possibly other organisms. If infection is dependent upon lack of normal echinochrome deposition, it may be that this pigment acts as a general disinfectant in this area which is especially open to invasion by micro-organisms.

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Microbial Insect Control--An Interdisciplinary Approach. Bohdan Maksymiuk, Forestry Sciences Laboratory, P. O. Box 887, Corvallis, Oregon.

An interdisciplinary approach is needed to accelerate science, technology, and practive of the microbial control. Diversified knowledge from the biological and physical sciences (such as insect pathology, entomology, chemistry, plant physiology, physics, meteorology, engineering, and biometrics) must be integrated and used in developing efficient microbial control methods.

Successful use of microbial insecticides depends primarily on the understanding of insect-pathogen-plant relationships, safe and suitable spray formulation, efficient spray equipment to obtain satisfactory plant coverage, and meaningful short- and long-range biological evaluation of field treatments.

A water-base spray formulation has been developed and successfully field-tested. This formulation contains fluorescent tracer for qualitative and quantitative spray deposit assessment on foliage and other substrata. Drop size spectra of this formulation, produced by conventional and nonconventional atomizing devices, were studied in the range from 100 to 500 microns mass median diameter. Basic knowledge from this study resulted in development of efficient spray equipment for aerial application of microbial insecticides.

Nucleopolyhedrosis virus and the Douglas-fir tussock moth larvae, Hemerocampa pseudotsugata, were used as test organisms in the helicopter spray tests to develop an operational method. In these field studies, it was found that application rate can be reduced tenfold (from 2.0 to 0.2 gallons per acre) using fine spray atomization of 106 microns mass median diameter without decreasing insect mortality. It has been demonstrated, for the first time, the feasibility of ultra-low volume application of microbial insecticides. This will result in the cost reduction and safety to aircraft especially in mountainous terrain.

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Comparisons between Different Pox-Like Virus Diseases in Insects. Max Bergoin and Constant Vago, Station de Recherches Cytopathologiques 30, 30, Saint-Christol/Montpellier-France.

Following the discovery, in our laboratory, of insect diseases caused by pox-like viruses, we described several cases of these "spindle diseases" in coleoptera: Melolontha melolontha L., Figulus sp. Phyllopertha horticola L. and Demodena boranensis BRUCH and in lepidoptera: Operophtera brumata HUBNER and Oreopsyche angustella H.S.

Comparative histopathology showed that in most cases two types of inclusions are present in the cytoplasm of infected adipose cells. One elongated, called spindles, others more or less spherical called spheroids. The spindles, regularly elongated in Melolontha and Phyllopertha, more rhembodedral with defined angles in Operophtera, Demodena and Figulus are rare in Oreopsyche. As revealed by electron microscopy, spindles are made of protein regularly disposed in a paracrystalline pattern. Spheroids large and only few per cell in Melolontha and Phyllopertha, are small and numerous in Operophtera and Figulus. In Figulus they are often cemented together and in Demodena they are lined with spindles by a dense substance. All the spheroids have a paracrystalline structure in thin sections and contain occluded virions.

The virions show a striking resemblance between each others and with those of the pox group. They are oval shaped in longitudinal sections and contain a dense unilaterally concave nucleoid delimited by a three-layered membrane. A filamentous structure is observed inside the dense material of the nucleoid. Surrounding the nucleoid a substance of less density forms a lenticular lateral body in its concavity. The coat of the virions is regularly bumped suggesting a mulberry-like structure of its surface.

The viral development was studied in three cases. It occurs in cytoplasmic virogenic areas, where different stages of immature virus particles could be distinguished. Mature virions are gradually occluded in crystallin protein.

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SESSION 5
Thursday Afternoon

Epizootiological Observations on the Microsporidoses of Heliothis Species and the Larval Parasites Campoletis perdistinctus and Cardiochiles nigriceps in North Carolina. Wayne M. Brooks, Department of Entomology, North Carolina State University, Raleigh, North Carolina.

An epizootiological study was undertaken on the microsporidian parasite Nosema heliothidis infecting the tobacco budworms Heliothis virescens and Heliothis zea. Weekly collections of Heliothis larvae on tobacco, corn and cotton were examined for incidence of microsporidian infection and parasitization by various larval parasites. In addition to presenting these data, observations on microsporidians discovered in the larval parasites C. perdistinctus and C. nigriceps will be discussed, especially in relation to N. heliothidis. Preliminary results on the effects of the various microsporidians on their hosts will also be presented.

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The Effect of Gamma Radiation on Growth Development and Mortality of Hemerocampa leucostigma. E. A. Hoffman and H. W. Rossmoore, Department of Biology, Wayne State University, Detroit, Michigan.

First and third instars and pupae of Hemerocampa leucostigma were radiated in a cesium rad cell at a dose rate of 10,000 R per hour. Larvae were weighed daily after irradiation, and head capsule diameters were measured following molt. Up to 10,000 R third instar larvae all survived to pupate. At doses above 10,000 R mortality is almost linear with dose. First instars were more radio-sensitive than third instars. Pupae survived 50,000 R as evidenced by 100 per cent emergence. There was no detectable effect on head capsule size at any dose level; however, there was a marked decrease in weight proportional to the radiation dose.

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The Fungus Metarrhizium anisopliae as a Pathogen of Mosquito Larvae. Donald W. Roberts, Boyce Thompson Institute for Plant Research, 1086 North Broadway, Yonkers, New York.

Larvae from a wide range of mosquito species have proved susceptible to the entomogenous fungus Metarrhizium anisopliae. Dose-response (based on time to 50 percent mortality) was directly related to the number of spores per cm^2 with floating spores, whereas with submerged spores mortality was delayed at high doses, apparently because the spores produced self-inhibitors. Larvae of all ages were susceptible, but older larvae succumbed more quickly than smaller ones; and large ($>500\ \mu$) aggregates of spores were less effective than small ($<125\ \mu$) aggregates. The modes of action included asphyxiation from spiracular infection and toxin production. Two toxins, destruxins A and B, were isolated from fungus-exposed larvae. The fungus holds some promise as a microbial control agent of mosquito larvae, but its final evaluation awaits field trials and studies of its influence on other members of the mosquito environment.

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Ultrastructural Observations on a Spirochetal Infection of the Brine Shrimp, Artemia salina. Greta E. Tyson, Department of Biological Structure, University of Washington, Seattle, Washington.

A microorganism displaying the ultrastructural features of a spirochete has been found in thin-sectioned tissues of the brine shrimp, Artemia salina (Linnaeus, 1758). Electron microscopical observations were made on a variety of tissues fixed in cacodylate-buffered glutaraldehyde and acrolein, post-fixed in osmium tetroxide, and embedded in Epon. Like all spirochetes so far examined with the electron microscope, the microbe from Artemia consists of three main parts: (a) a slender protoplasmic cylinder (average diameter, 0.18u), (b) axial fibrils (approximately 150 A in diameter) which are twined around the protoplast, and (c) an outer envelope or sheath, enclosing both protoplasmic cylinder and axial fibrils. The number of axial fibrils evident in cross-sectional profiles of the spirochete in Artemia varied from 0 to 4, with 2 fibrils being the number most frequently seen. Spirochetes have been found in the cytoplasm of cells from all three regions of the maxillary gland (end-sac, efferent tubule, and terminal duct), as well as in the cytoplasm of hypodermal, muscle, and blood cells. Spirochetes also occur extracellularly and may be very abundant in haemocoelic spaces and in the lumen of the end-sac of the maxillary gland. (Supported by USPHS postdoctoral fellowship 5 FO2 GM25538-02 and USPHS research grant HE02698.)

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The Advantages of Pathological and Oncological Research with Invertebrates. Hans E. Kaiser, Department of Anatomy, School of Medicine, The George Washington University, Washington, D. C.

According to the fossil record true tissues must have developed earlier in invertebrates than in plants, i.e., during Pre-Cambrian time, whereas plant tissues could not have appeared much earlier than in the first Psilophytales, in late Silurian or Devonian times (Kaiser, 1968). Neoplasms also, as a sign of cellular disorder, depend for their malignant existence on the special characteristics of the host tissue structures for their infiltrative or metastatic growth. Our present investigations are concerned with the question of the development of the various characteristics of malignant growth which are cellular in type and those which could not have developed before the occurrence of true tissues. This is the reason why comparisons between protozoans and lower invertebrates and vertebrates are so significant for comparative pathology and oncology. It is possible to compare certain characteristics of neoplastic cells of true tissues with those cells of unicellular organisms or organisms without true tissues, such as Porifera. It is unrealistic to try to establish direct similarities between those cells of organisms of different morphology, namely those of unicellular organisms, cell colonies, organisms without true tissues and different types of organisms with true tissues. This holds true for animals as well as plants. Since we are able to produce true neoplasms with mammalian carcinogens in higher invertebrates, such as molluscs, further investigations with lower invertebrates on the development of neoplasms are even more important.

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Histologic Incomparabilities of Specific Characteristic Plant and Animal Tissues. Hans E. Kaiser, Department of Anatomy, School of Medicine, The George Washington University, Washington, D. C.

Cells of organisms of both kingdoms have already been compared over the past several years. Most cytologic components are comparable with the exception of the cell wall and organelles such as the plastids. The same holds true for chemical compounds in their distribution in plants and animals. The comparability of animal and plant tissues is further confused by the dissimilarity of certain structures. For example, the supporting structures in certain plants should not be compared to animal connective tissue with regard to their ontogenetic origin, nor should the structures of the fluid distribution as vessels or sieve tubes. In accordance with the author's investigations (reported in earlier publications) concerning the comparability of histological structures, it now seems appropriate to discuss the incomparability of these systems. In this regard we must distinguish between the phylogenetic, ontogenetic, morphologic or functional approach. It is essential to basic concepts of comparative pathology and the pathology of invertebrates, to establish a proper relationship between the pathology of plants on the one hand and that of animals on the other. It should be pointed out, however, that on a histologic basis this problem is grossly neglected in current research efforts.

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Mitoses in Thin Sections of Asexual Hydras. Carlton H. Nadolney, Department of Pathology, Beekman-Downtown Hospital and Department of Biology, Pace College, New York, New York.

Extended specimens of asexual Hydra littoralis var. Loomis and H. sp. var. Bryden laboratory strains were fixed in Dalton's chromeosmium solution at intervals after feeding, embedded in methacrylate, and sectioned serially at 1 micron, using an Ultramicrotome with glass knives. Typical mitoses were demonstrated with the Turchini phenylxanthenone reaction, gallocyanin-chromalum stain, methyl green-pyronin Y stain, Feulgen reaction, and azure B bromide method. Staining methods for polysaccharides were also useful.

The cytological characteristics of the body-wall were studied in the tentacular, hypostomal, gastric, penduncular, and basal disc regions. The topological results were correlated in a cytophysiological interpretation of the dynamics of the hydra body column. A higher incidence of mitoses than reported generally was observed in the epidermis and gastroderm, and was related to active biosynthetic and metabolic processes. These metabolic processes reflect a broad gradient of growth and differentiation in the body column, one which decreases aborally.

In the epidermis, epithelio-muscular cells have frequent mitoses in the hypostomal, gastric, and peduncular regions. The interstitial cells have a high mitotic coefficient, with several cells in a given cluster at the same division stage. These have been described as totipotent cells capable of differentiation into other specialized cell types of both epithelial layers and of forming gametes.

Mitotic activity was often seen in all regions of the gastroderm, except in the distal tentacles and basal disc. The identity of these cells was not always clear, but some were digestive cells and possibly others mucous exocrine gland cells.

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The Fine Structure of Some of the Developmental Stages of Mattesia grandis McLaughlin (Sporozoa, Neogregarinida) Parasite of the Boll Weevil Anthonomus grandis Boheman. Jiri Vavra, College of Veterinary Medicine, University of Illinois, Urbana, Illinois, and Roy E. McLaughlin, USDA, Boll Weevil Research Laboratory, State College, Mississippi.

Several developmental stages (sporozoites, macronuclear schizonts and merozoites, gamonts) have been examined using an electron microscope. A conoidal apparatus, consisting of conoid, apical ring and subpellicular fibres was present in all of these stages. The conoidal apparatus was similar in structure to the same organelle of other sporozoa.

In young schizonts (one to four nuclei) of the second (macronuclear) schizogony the conoidal apparatus is transformed into an organelle similar to the mucron of some eugregarines. This mucron consists of a specialized area of the cell membrane from which fine fibres are running into a large vacuole situated directly under the cell membrane. The top part of the vacuole is encircled by two ring-like structures formed by the dilatation of the original conoid and apical ring. The mucron may be involved in the nutrition of the organism as its vacuole is partly filled by anastomosing protrusions of the cytoplasm.

The mucron disappears when the schizont reaches the multinucleate state. Later the merozoites budd from the surface of the schizont in a manner similar to coccidia. Each merozoite again has the conoid and accompanying structures. The conoidal apparatus of the merozoite persists thru the gamont stage, usually serving as the point of contact between 2 gamonts during their pairing.

The presence of conoidal apparatus thru an important part of the life cycle of Mattesia, its transformation into a mucron together with the mode of formation of merozoites indicate that the neogregarinida combine the fine structure characters of both the eugregarinida and the eucoccida, thereby suggesting the actual phylogenetic relationship between these sporozoans with the neogregarines as a link between eugregarines and coccidia.

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Addendum: late Abstract

A Progress Report on a New Coccidian in a Beetle Larva (Trogoderma parabile). Lydia Tan and H.E. Welch, Department of Zoology, University of Manitoba, Winnipeg, Canada.

An Adelina sp. a Coccidian was found in the grain beetle larvae, Trogoderma parabile, Beal. The main seat of infection appears to be the fat bodies of the host. The developmental stages such as the zygote, mature oocysts with sporocysts, sporozoites, merozoites and gametocytes have been observed under the phase microscope. Ultrastructural studies on these stages are also now being carried out.

SOCIETY FOR INVERTEBRATE PATHOLOGY

The founding of this Society on May 9, 1967, at the University of Washington, Seattle, represented the successful convergence of the interests of scientists of several disciplines and several nationalities. Insect pathologists were aware of their own common interests, but soon after the publication in the 1960's of the Journal of Insect Pathology under the editorship of Dr. E.A. Steinhaus, Irvine, California, they became aware of an equally large group of pathologists concerned with molluscs, crustaceans, and other invertebrates. Both groups quickly recongnized their mutual problems and interests. The establishment of the Society, therefore, brought together these two diverse groups and bridged international boundaries to link Asian, European, and North American scientists.

This variety of disciplines and geographical interests played a significant role in the organization of the Society, and particularly in shaping its objectives. These include the promotion of scientific knowledge and investigations of the diseases of invertebrate animals through meetings, symposia, and publications; the planning, organization, and administration of projects; and especially the development of international co-operation in the attainment of these objectives.

From the organizing committee of twelve members has grown, in the short period of two years, a Society of 410 members. This includes one Honorary member, 79 Founding members, 113 Charter members, 184 Regular members, and 33 Student members. That the Society has prospered so well has been largely due to the organizational abilities of its Presidents, Dr. E.A. Steinhaus, Dr. A. Sparks, Dr. C. Vago (France), its Secretary Dr. A. Heimpel and to Dr. Victor Sprague, its A.I.B.S. representative.

The international flavour is detected in the membership list which shows over one third of the membership from outside of North America and representing 32 countries.

The official publishing organ of the Society is the Journal of Invertebrate Pathology. This journal is published by Academic Press, New York, and is available to members by subscription with their dues. A quarterly newsletter, the S.I.P. Newsletter, is in its second volume and covers a wide variety of topics. A membership list has been distributed and a second list will be available in early 1970.

Meetings are held annually. The first was in Columbus, Ohio, and the second will be in Burlington, Vermont in 1969; both were under the auspices of the A.I.B.S. The third annual meeting in 1970 will be in conjunction with the International Colloquium of Insect Pathology and Biological Control in Washington, D.C., in 1970. The fourth meeting will be held outside of North America.

The by-laws permit Divisions of the Society which are based on professional interests, and more recently changes in the by-laws may permit local branches. These two mechanisms will encourage regional meetings and activities.

The Society is an adherent society of the A.I.B.S., and is currently considering membership in the International Council of Societies of Pathology. The International Society of Bee Pathologists has recently joined the Society. Joint meetings with the Society for Experimental Pathology are also being considered.

Over the last two years the activities of the Society were largely organizational. Two projects have been started and include a Placement Service and a catalogue of viruses and microorganisms pathogenic in invertebrates. The Society will also prepare a brochure describing career opportunities in inverte-

brate pathology for high school and undergraduate university science students.

The Society's Executive includes:

President:	Dr. A.K. Sparks, College of Fisheries, University of Washington, Seattle, Washington 98105, U.S.A.
Vice-President:	Dr. Constant Vago, Faculty of Sciences, University of Montpellier, France.
Past-President:	Dr. E.A. Steinhaus, Center of Pathobiology, University of California, Irvine, California 92664, U.S.A.
Secretary:	Dr. H.E. Welch, Department of Zoology, University of Manitoba, Winnipeg, Canada.