Program of The American Society of Parasitologists

THE AMERICAN SOCIETY OF PARASITOLOGISTS

PROGRAM AND ABSTRACTS
of the
FORTY-FOURTH
ANNUAL MEETING*

SHOREHAM HOTEL
WASHINGTON, D.C.
NOVEMBER 3 - 7, 1969

NOTES

1. Registration will take place in the Lower Lobby.

2. Ladies Lounge will be located in the Heritage Room. Coffee will be available.

3. Military Service Reserve credit will be arranged at a desk in the Lower Lobby.

* Held jointly with the American Society of Tropical Medicine and Hygiene.

1969
PROGRAM

ASP HEADQUARTERS

Tuesday through Friday the Press Room will serve as the Headquarters for the American Society of Parasitologists.

Monday Afternoon - 3 Nov. - 1:30 PM
Council Room

Council Meeting: Officers, Chairmen of Committees and Past Presidents may attend.
If necessary an evening session will be convened at 7:30 PM.

Monday Evening - 3 Nov. - 8:00 PM
Tudor Room

Conference on "Parasites as Biological Tags for Marine Animals" under the sponsorship of the International Biological Program Committee of the American Society of Parasitologists.

Elmer Noble, University of California, Santa Barbara
and
Carl J. Sindermann, Tropical Atlantic Biological Laboratory, Bureau of Commercial Fisheries, Miami, Florida,
Presiding

Interested persons are invited.

Tuesday Morning - Session 1 - 4 Nov. - 8:30 AM
Diplomat Room

PHYSIOLOGY AND BIOCHEMISTRY - CESTODA
Theodor Von Brand
Laboratory of Parasitic Diseases, N.I.H.,
Bethesda, Maryland, Presiding

1. Intestinal Starch Digestion and Absorption in Rats Infected with Hymenolepis diminuta. ROBERT W. MEAD and LARRY S. ROBERTS, University of Massachusetts, Amherst.

2. Organism Weight and Tissue Lipids of Rat Host and Hymenolepis diminuta following Dietary Lipid Alteration. CHAUNCEY G. GOODCHILD, Emory University, Atlanta, Georgia and ARDIS L. CRAMER, Agnes Scott College, Decatur, Georgia.

3. Changes in the Amino Acid Pool of Hymenolepis diminuta (Cestoda) Associated with Induced Imbalance of Exogenous Amino Acids. LESLIE H. CHAPPELL and CLARK P. READ, Rice University, Houston, Texas.

4. Tegumentary Phosphatases of Hymenolepis diminuta. SUE CARLISLE DIKE and CLARK P. READ, Rice University, Houston, Texas.

5. Effects of Thymine and Thymine Analogs on the *in vitro* Uptake of Uracil-2-C^{14} by Hymenolepis diminuta. AUSTIN J. MACINNIS and ROBERT K. RIDLEY, The University of California, Los Angeles.

6. Characterization of Hymenolepis diminuta DNA. CLINT E. CARTER, AUSTIN J. MACINNIS and ROBERT K. RIDLEY, The University of California, Los Angeles.

7. Mitotic Activity and Thymidine Incorporation in the Germinative Region of Developing Hymenolepis diminuta. R. I. BOLLA and L. S. ROBERTS, University of Massachusetts, Amherst.

8. Measurements of the Genetic Basis of Parasitic Reduction. DENNIS G. SEARCY and AUSTIN J. MACINNIS, The University of California, Los Angeles.

9. Effects of Ultraviolet Light on Hymenolepis microstoma. J. H. BUTLER and F. H. WHITTAKER, University of Louisville, Kentucky.

10. Gamma Radiation-Induced Effects on Genetic Fitness of Hymenolepis nana. PAUL H. RODRIGUEZ and J. W. CRENSHAW, University of Rhode Island, Kingston.

11. Cytochemical Observations on the Mitochondrial Oxidation of 3-3' diaminobenzidine (DAB). RICHARD D. LUMSDEN, JOHN A. OAKS and RICHARD R. MILLS, Tulane University, New Orleans, Louisiana.

12. a-Glycerophosphate Oxidase of Taenia taeniaeformis. EUGENE C. WEINBACH and THEODOR VON BRAND, National Institute of Allergy and Infectious Diseases, N.I.H., Bethesda, Maryland.

13. Biosynthesis de novo of Purines and Pyrimidines in Mesocestoides (Cestoda). ROBERT L. HEATH, The University of California, Los Angeles, and JOSEPH L. HART, Merrimack College, North Andover, Massachusetts.

14. The Action of Bile Salts on Hymenolepidid Cystecercoid Hatching. RICHARD D. TKACHUCK, The University of California, Los Angeles.

Tuesday Morning - Session 2 - 4 Nov. - 8:30 AM

Tudor Room

GENERAL - TAXONOMY AND MORPHOLOGY

Robert E. Kuntz
Southwest Foundation for Research and Education,
San Antonio, Texas, Presiding

15. System for Determining Percent Survival of Entamoeba histolytica cysts in vitro. RICHARD STRINGER, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland.

16. Development of Elongate Gametocytes of Leucocytozoon simondi in Erythroblasts in the Bone Marrow of White Pekin Ducklings. A. L. AGER and R. M. CORWIN, Michigan State University, East Lansing.

17. Plasmodium tenue Laveran and Marullaz, 1914. REGINALD D. MANWELL, Syracuse University, Syracuse, New York.

18. Attachment-Organ Development of Cotylurus variegatus in Larus delawarensis. M. E. HAIGHT and D. N. JENSEN, McMaster University, Hamilton, Ontario, Canada.

19. Host and Organ Specificity of the Metacercarial Cysts of the Genus Ascocotyle Looss, 1899. P. C. STEIN, Southern University in New Orleans and Tulane University and R. D. LUMSDEN, Tulane University, New Orleans, Louisiana.

20. Intraspecific Variation of Quinqueserialis quinqueserialis in Rodent Hosts. JOHN M. KINSELLA, University of Montana, Missoula, Montana.

21. Basic Hook Musculature and Movements of Invasive Oncospheres from Hymenolepis diminuta. ROBERT E. OGREN, Wilkes College, Wilkes-Barre, Pennsylvania.

22. Morphology and Histochemistry of Genital Cone of Cooperia punctata and Comparison of Seven Cooperia Species. FRANK STRINGFELLOW, USDA, ARS, Beltsville Parasitological Laboratory, Beltsville, Maryland.

23. "Pearl-like nodules" in the Macaque Monkeys. M. M. WONG and H. D. CONRAD, National Center for Primate Biology, University of California, Davis.

24. A Comparison of Haematopinus eurysternus from New Mexico and H. quadripertusus from Puerto Rico. WILLIAM P. MELENEY, USDA, Albuquerque, New Mexico.

25. Preliminary Taxonomic Value of Antennal Sensilla of the Anoplura. FREDERICK H. MILLER, Meadowbrook Hospital, East Meadow, New York.

26. Lesions in Capra hircus due to Demodex caprae. W. B. NUTTING and R. R. LEBEL, University of Massachusetts, Amherst.

27. Demodex caprae - Structural Features of Use in Demodicid Systematics. C. E. DESCH and W. B. NUTTING, University of Massachusetts, Amherst.

28. How Many Species Has Salmincola, a Genus of Copepods Parasitic on Fishes? Z. KABATA, Fisheries Research Board of Canada, Biological Station, Nanaimo, British Columbia, Canada.

Tuesday Afternoon - Session 3 - 4 Nov. - 1:50 PM
Diplomat Room

PHYSIOLOGY AND BIOCHEMISTRY - PROTOZOA
Quentin M. Geiman
Stanford University School of Medicine,
Palo Alto, California, Presiding

29. Monofactorial Inheritance of Susceptibility of Aedes aegypti to Plasmodium gallinaceum. W. L. KILAMA and G. B. CRAIG, JR., University of Notre Dame, Notre Dame, Indiana.

30. Immersion Interferometry of Plasmodium berghei and Plasmodium vinckei. MICHAEL J. AUTUORI and C. W. LACAILLADE, St. John's University, Jamaica, New.York.

31. Folate Metabolism in Plasmodium lophurae. EDWARD G. PLATZER, Rockefeller University, New York, New York.

32. Vital Considerations Concerning Purines, Pyrimidines and Amino Acid Incorporation into Nucleic Acids and Proteins of Plasmodium berghei. C. LANTZ, K. VAN DYKE and L. H. SAXE, West Virginia University Medical Center, Morgantown.

33. Phagocytic Activity of Splenic Macrophages in Rodent Malaria. JOYCE S. CHOW and JULIUS P. KREIER, Ohio State University, Columbus.

34. Maltose Metabolism of Trichomonas gallinae (Rivolta, 1878). JAMES J. DALY, University of Arkansas Medical Center, Little Rock.

35. Effects of Catecholamines on the Parasitemia Levels of Trypanosome rotatorium in Rana clamitans. G. MASON and J. R. SEED, Tulane University, New Orleans, Louisiana.

36. Biochemical Changes in Livers of Guinea Pigs Infected with Trypanosoma gambiense. Y. MARCIACQ and J. R. SEED, Tulane University, New Orleans, Louisiana.

37. Thymidine Kinase in Culture and Bloodstream Forms of Trypanosoma (Trypanozoon) rhodensiense. P. L. CHELLO and J. J. JAFFE, College of Medicine, University of Vermont, Burlington.

38. Isolation and Properties of Phosphohexose Isomerase from Trypanosoma cruzi. E. L. RISBY, Meharry Medical College, Nashville, Tennessee.

39. Cytochemical Identification of b-galactosidase, Adolase, Phosphorylase, and Lipids in Eimeria stiedae. JOHN C. FRANDSEN, USDA Regional Parasite Research Laboratory, Auburn, Alabama.

40. Economic Significance of Coccidiosis in Calves. PAUL R. FITZGERALD and M. E. MANSFIELD, College of Veterinary Medicine, University of Illinois, Urbana.

41. Toxoplasma gondii: DNA Biosynthesis and Characterization. A. GELDERMAN, J. PERROTTO and M. LUNDE, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland.

42. Progress Report on the International Clearing House Program for Parasitological Material. FREDERICK H. MILLER and IRVING ABRAHAMS, Meadowbrook Hospital, East Meadow, New York.

Tuesday Afternoon - Session 4 - 4 Nov. - 1:50 PM
Tudor Room

GENERAL - NEMATODA
Franklin G. Wallace
University of Minnesota, Presiding

43. Wildlife Reservoirs of Capillaria hepatica (Bancroft, 1893). GENE B. SOLOMON, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, and CHARLES O. HANDLEY, JR., Division of Mammals, Smithsonian Institution, Washington, D. C.

44. Distribution of Meningeal Worm (Pneumostrongylus tenuis) in the Southeastern United States. ANNIE K. PRESTWOOD and JAMES F. SMITH, School of Veterinary Medicine, University of Georgia, Athens.

45. Influence of Weather on Intensity of Infection of Bighorn Sheep with Lungworms of the Genus Protostrongylus. DONALD J. FORRESTER, University of Florida, Gainesville.

46. Skrjabinema ovis from Angora Goats in Texas. MARVIN LADERMAN and V. J. THEODORIDES, Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

47. Food Attraction of Coprophagous Beetles Serving as Intermediate Hosts for Spirurids of Swine. G. T. FINCHER and T. B. STEWARD, Georgia Coastal Plain Experiment Station, Tifton, Georgia.

48. Effect of Stocking Rates on Parasitism of Steers on Continuously vs. Rotationally Grazed Winter Temporary Pasture. H. CIORDIA, USDA Regional Parasite Research Laboratory, Experiment, Georgia.

49. Develoopment, Migration and Survival on Pasture of Gastrointestinal Nematodes of Cattle - Summer Contamination. AARON GOLDBERG, ADP, ARS, USDA, Beltsville Parasitological Laboratory, Beltsville, Maryland.

50. Effects of Repeated Desiccation and Hydration on Infective Haemonchus contortus Larvae. KENNETH S. TODD, JR., NORMAN D. LEVINE and CAROLE C. WHITESIDE, College of Veterinary Medicine, University of Illinois, Urbana.

51. Evaluation of the Baermann Technic for Infective Larvae of Haemonchus contortus. NORMAN D. LEVINE, KENNETH S. TODD, JR. and CAROLE C. WHITESIDE, College of Veterinary Medicine, University of Illinois, Urbana.

52. Anatrichosoma spp. in the Old World Non-human Primates. HELEN DAVIS CONRAD and MING M. WONG, National Center for Primate Biology, University of California, Davis.

53. Anatrichosoma Infection in African Monkeys. THOMAS C. ORIHEL, Delta Regional Primate Research Center, Covington, Louisiana.

54. Further Studies on Filariasis in Dogs as Determined through Blood Studies. R. N. GARCIA, J. W. WARD and R. P. DODDS, University of Mississippi School of Medicine, Jackson.

55. Survey of Helminth Parasites of Dogs from Brazos County, Texas. J. O. COSTA, T. J. GALVIN and R. R. BELL, Texas A&M University, College Station.

56. Geographical Distribution and Seasonal Incidence in the Helminth Parasites of the Starling, Sturnus vulgaris L. JAY D. HAIR, University of Alberta, Edmonton, Alberta, Canada.

Tuesday Evening - Session 5 - 4 Nov. - 8:00 PM
Diplomat Room

ASP - ASTMH SYMPOSIUM ON
THE TEACHING OF PARASITIC DISEASES IN MEDICAL SCHOOL
Harold W. Brown
Columbia University, Presiding

57. The Teaching of Parasitic Diseases: Then and Now. K. L. HUSSEY, Columbia University, New York, New York.

58. Experimental Parasitology and Tropical Medicine at Brown. ALFRED W. SENFT, Brown University, Providence, Rhode Island.

59. Influence of Morphology and Ecology of the Second-Year Medical Student on the Knowledge-Transport System. DONALD V. MOORE, University of Texas Southwestern Medical School, Dallas.

60. Parasitic Diseases Teaching Experiences in Medical School. JOHN H. CROSS, NAMRU No. 2, APO San Francisco.

61. Parasitic Diseases a Part of Medicine. HAROLD W. BROWN, Columbia University, New York, New York.

DISCUSSANTS

Jerrold A. Turner, UCLA School of Medicine, Los Angeles
Harry Most, New York University, New York, New York
J. Clyde Swartzwelder, LSU School of Medicine, New Orleans, Louisiana
Donald Heyneman, University of California, San Francisco
Edward K. Markell, Permanente Medical Group, Los Angeles, California

Each discussant is limited to five minutes.

Wednesday Morning - Session 6 - 5 Nov. - 8:30 AM
Diplomat Room

GENERAL - HELMINTH PATHOLOGY
George W. Luttermoser
Research Grants Division, NIH, Bethesda, Maryland,
Presiding

62. Junctional Nodules in Schistosomiasis: Development of Granulomatous Nodules at the Mesenteric Attachment to the Bowel. B. H. KEAN and DAVID T. DENNIS, Cornell University Medical College, New York, New York.

63. Experimental Infections with Schistosoma haematobium in the Chimpanzee. E. H. SADUN, WRAIR, Washington, D. C., F. VON LICHTENBERG, Harvard Medical School, Boston, Massachusetts, A. W. CHEEVER, NIAID, NIH, Bethesda, Maryland, D. G. ERICKSON and R. L. HICKMAN, WRAIR, Washington, D. C.

64. On the Evolution and Involution of Egg Lesions in the Liver of Horses Infected with Schistosoma japonicum. H. F. HSU, S. Y. HSU, J. R. DAVIS and W. MERGNER, University of Iowa, Iowa City and University of Arizona, Tucson.

65. Cytology of the Bile Duct in Mice Infected with the Tapeworm Hymenolepis microstoma. DANIEL S. KARIN and RICHARD D. LUMSDEN, Tulane University, New Orleans, Louisiana.

66. Pathogenicity of Geographic Strains of Angiostrongylus cantonensis in the Taiwan Monkey. J. H. CROSS and J. W. FRESH, NAMRU No. 2, APO San Francisco.

67. Pulmonary Arterial Changes in Canine Dirofilariasis. SI-KWANG LIU, DALE A. YARNS and ROBERT J. TASHJIAN, The Animal Medical Center, New York, New York.

68. Lymphatic Involvement in Experimental Filariasis. DONALD L. PRICE, Biodynamics Research Corporation, Rockville, Maryland and JAMES M. MORRIS, Armed Forces Institute of Pathology, Washington, D. C.

69. Feeding Behavior of Adult Ancylostoma caninum and Related Blood Loss in the Host. ULRICH P. KALKOFEN, School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana.

70. The Effect of Trichospirura leptostoma on the Pancreas of Callithrix jacchus. W. N. SMITH and B. M. LEVY, The University of Texas Dental Science Institute, Houston.

Wednesday Morning - Session 7 - 5 Nov. - 8:30 AM

Tudor Room

GENERAL - NEMATODA
Thomas C. Orihel
Delta Regional Primate Research Center,
Covington, Louisiana, Presiding

71. Susceptibility of Several Insect Pests to Neoaplectana glaseri. CHARLES P. TURCO, Lamar State College of Technology, Beaumont, Texas and SEWELL H. HOPKINS and WALTER H. THAMES JR., Texas A & M University, College Station.

72. An Abnormality of Nematode Cuticle. G. J. JACKSON, M. A. RUDZINSKA and D. D. DESPOMMIER, The Rockefeller University, New York, New York.

73. Dog Infection with Ancylostoma caninum: Comparison of Prepatent Period Following Infection of Puppies by Oral and Percutaneous Routes. WILLIAM M. STONE, Diagnostic and Research Laboratories, School of Veterinary Medicine, University of Georgia, Tifton.

74. Migration Patterns and Development of Metastrongyloids in Carnivores. P. H. G. STOCKDALE and ROY C. ANDERSON, University of Guelph, Guelph, Ontario, Canada.

75. Arrested Development of Haemonchus contortus in Lambs. N. M. BLITZ and H. C. GIBBS, Institute of Parasitology, Macdonald College, P. Q., Canada.

76. Larval Development and Transmission of *Parafilaroides decorus* (Nematoda: Pseudaliidae) in the California Sea Lion (*Zalophus californianus*). MURRAY D. DAILEY, California State College at Long Beach, Long Beach.

77. Relation of Sex Balance of *Angiostrongylus cantonensis* to Location of Worms and to Lesions in Lungs of Rats. ELSA L. WINSOR, School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana.

78. Infection of Ticks with *Dipetalonema viteae*. GUILLERMO PACHECO, MARK J. ATKINS and JOAN GURIAN, NIAID, NIH, Bethesda, Maryland.

79. Observations on the Development of *Brugia pahangi* in Small Laboratory Animals. LAWRENCE R. ASH and JOHN M. RILEY, School of Public Health, University of California at Los Angeles.

Wednesday Morning - Session 8 - 5 Nov. - 11:00 AM

Diplomat Room

PRESIDENTIAL ADDRESS
American Society of Parasitologists
Paul P. Weinstein
University of Notre Dame, South Bend, Indiana,
Presiding

80. An Old Timer's Look at Parasite Physiology

THEODOR VON BRAND
Formerly Head, Section on Physiology and Biochemistry, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland.

Wednesday Noon - 5 Nov. - 12:15 PM
Blue Room

ANNUAL LUNCHEON AND BUSINESS MEETING
Theodor von Brand, Presiding

Remainder of the afternoon is free for sightseeing, institutional visits, etc. See joint program for details.

Wednesday Evening - Session 9 - 5 Nov. - 8:00 PM
Diplomat Room

ASP SYMPOSIUM ON
TWENTY YEARS PROGRESS IN PARASITE PHYSIOLOGY AND BIOCHEMISTRY
Dedicated to Dr. Theodor von Brand
Paul P. Weinstein
University of Notre Dame, Presiding

81. Introduction. PAUL P. WEINSTEIN, University of Notre Dame, South Bend, Indiana.

82. Physiology and Biochemistry of Parasitic Protozoa. WILLIAM TRAGER, The Rockefeller University, New York, New York.

83. Comparative Energy Metabolisms of some Parasitic Helminths. HOWARD J. SAZ, University of Notre Dame, South Bend, Indiana.

84. Physiology and Biochemical Aspects of the Host-Parasite Relation. CLARK P. READ, Rice University, Houston, Texas.

85. Summation. ERNEST BUEDING, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

Thursday Morning - Session 10 - 6 Nov. - 8:30 AM
Diplomat Room

IMMUNOLOGY AND SEROLOGY - TREMATODA, CESTODA AND NEMATODA
Elvio H. Sadun
Walter Reed Army Institute of Research, Washington, D. C.,
Presiding

86. Immunologic Studies on Schistosomiasis in Rhesus (Macaca mulata) Monkeys. SHIRLEY E. MADDISON, SADIE J. GEIGER and IRVING G. KAGAN, National Communicable Disease Center, Atlanta, Georgia.

87. Nature of the Reaction of Antigen with Sensitized Lymphocyte-Dependent Release of Histamine from Platelets of Rabbits Infected with Schistosoma mansoni. J. F. BARBARO and M. J. SCHOENBECHLER, WRAIR, Washington, D. C.

88. Immunization of Rhesus Monkeys against Schistosome Infection by Cercariae Exposed to High Doses of X-radiation. S. Y. LI HSU, H. F. HSU and J. W. OSBORNE, University of Iowa, Iowa City.

89. Evidence of Indirect Antagonism by Schistosoma mansoni Against Development of the Echinostome, Paryphostomum segregatum, within the Snail Host. HO KAN LIM and DONALD HEYNEMAN, Hooper Foundation, University of California Medical Center, San Francisco.

90. On the Course of Infection by the Schistosome Trematode Trichobilharzia ocellata in its Duck Hosts as Indicated by Parasite Egg Passage. T. K. R. BOURNS, M. E. RAU and J. C. ELLIS, University of Western Ontario, London, Canada.

91. Development of Trichobilharzia ocellata (Schistosomatidae) in Primary and Challenge Infections in Anas rubripes. J. C. ELLIS, T. K. R. BOURNS and M. E. RAU, University of Western Ontario, London, Canada.

92. Resistance of the Black Duck Anas rubripes to Reinfection by the Schistosome Trichobilharzia ocellata. M. E. RAU, T. K. R. BOURNS and J. C. ELLIS, University of Western Ontario, London, Canada.

93. Host Response of the Flour Beetle, Tribolium confusum, to Single and Challenge Infection with Hymenolepis diminuta and H. microstoma. DONALD HEYNEMAN, University of California, San Francisco, and MARIETTA VOGE, University of California, Los Angeles.

94. Immunogenicity of Ascaris suum Larval Metabolic and Somatic Antigens. JORGE GUERRERO and PAUL H. SILVERMAN, University of Illinois, Urbana.

95. Lymohocyte and Macrophage Population Kinetics in Ascariasis. E. J. L. SOULSBY, University of Pennsylvania, Philadelphia.

96. Sequelae to Intravenous and Subsequent Oral Inoculation with Oesophagostomum radiatum in Cattle. HARRY HERLICH, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

97. Hemoglobin Types as an Indicator of Resistance to Haemonchus contortus Infection in Sheep. R. E. BRADLEY and A. F. JILEK, University of Florida, Gainesville.

98. Trichinella spiralis Infections in Neonatally Thymectomized Rats. R. W. GORE, H. J. BURGER and E. H. SADUN, WRAIR, Washington, D. C.

99. Cell-mediated Immunity in Rats Infected with Trichinella spiralis. H. J. BURGER, R. W. GORE and E. H. SADUN, WRAIR, Washington, D. C.

100. Transformation of Lymohocytes from Animals Sensitized to Trichinella spiralis. CHARLES W. KIM, MAHENDRA P. JAMUAR and L. D. HAMILTON, Brookhaven National Laboratory, New York, New York.

Thursday Morning - Session 11 - 6 Nov. - 8:30 AM
Tudor Room

GENERAL - PROTOZOA, TREMATODA AND NEMATODA
Martin J. Ulmer
Iowa State University, Ames, Presiding

101. Isolation of Naegleria gruberi from Nasal Swab of a Healthy Individual. G. HEALY, J. SHUMAKER, F. PAGE, D. ENGLISH and M. SCHULTZ, National Communicable Disease Center, Atlanta, Georgia and University of Wisconsin, Janesville.

102. A Plasmodium similar to P. mexicanum in Arizona Lizards. SAM R. TELFORD, JR., Gorgas Memorial Laboratory, Panama, Republic of Panama.

103. Repetitive Transmission of P. berghei yoelii in an A. stephensi-Mouse System. LIONEL T. RICHARD, MAURICE E. KING and ALAN M. SHEFNER, IIT Research Institute, Chicago, Illinois.

104. Characteristics of Sporozoite-Induced Plasmodium berghei yoelii Infections in Mice after 46 to 72 Hours Incubation. MORRIS D. SCHNEIDER, MAURICE E. KING, LIONEL T. RICHARD, NELLA SEAL and ALAN M. SHEFNER, IIT Research Institute, Chicago, Illinois.

105. Schizogonic Cycles of Leucocytozoon dubreuili and L. fringillinarum. R. A. KHAN, University of Toronto, Ontario, Canada.

106. Simulium innocens a New Vector of Leucocytozoon simondi in Canada Geese (Diptera: Simuliidae). I. BARRY TARSHIS, Patuxent Wildlife Research Center, Laurel, Maryland.

107. Transfer of a Hemogregarine from Boa constrictor to a Lizard, Anolis carolinensis, by a Mosquito Vector. THEODORE BOODEN, JOWETT CHAO and GORDON H. BALL, University of California, Los Angeles.

108. Pathway and Timing of Invasion of Sporozoites of Eimeria stiedae (Lindemann, 1865). ROBERT L. SLATER, M. A. QUISENBERRY and P. R. FITZGERALD, University of Illinois, Urbana.

109. Incidence of Several Species of Chicken Coccidia in Some Poultry Raising Areas of the United States. R. L. KENNETT, JR., G. T. WANG and S. KANTOR, American Cyanamid Company, Agricultural Division, Princeton, New Jersey.

110. Role of a Hyperparasite (Urosporidium) in the Dispersal of Microphallid Metacercariae from the Blue Crab. JOHN A COUCH and MARTIN W. NEWMAN, Fish and Wildlife Service, Biological Laboratory, Oxford, Maryland.

111. The Trematode, Aponurus sp., and its Definitive Host, the Deepsea Smelt, Leuroglossus stilbius. ELMER R. NOBLE and JUDITH D. ORIAS, University of California, Santa Barbara.

112. Fertilization of Paragonimus westermani in Experimental Animals. P. C. FAN, Department of Medical Biomorphics, National Defense Medical Center and C. H. CHIANG, Parasitology Laboratory, Department of Medical Research, Veterans General Hospital, VACRS, Taipei, Taiwan, Republic of China.

113. Cephalopods as Intermediate Hosts for Larval Didymozoids. F. G. HOCHBERG, JR., University of California, Santa Barbara.

114. Re-Investigation of Trichinella spiralis Life Cycle. WIESLAW J. KOZEK, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana.

Thursday Afternoon - Session 12 - 6 Nov. - 1:50 PM
Diplomat Room

PHYSIOLOGY AND BIOCHEMISTRY - TREMATODA, NEMATODA, ACANTHOCEPHALA AND ARTHROPODA
Austin J. MacInnis
University of California, Los Angeles, Presiding

115. The Role of Ingested Hemoglobin in the Nutrition of Schistosoma mansoni. R. A. ZUSSMAN, P. M. BAUMAN and J. C. PETRUSKA, Abbott Laboratories, Inc., North Chicago, Illinois

116. Effects of NaCl and Glucose on Longevity of Miracidia and Cercariae of Schistosoma mansoni. HAROLD L. ASCH and D. V. MOORE, University of Texas Southwestern Medical School, Dallas.

117. Rate of Destruction of Schistosoma mansoni Eggs in the Tissues of Mice. LOREN A. ANDERSON, Tulane University, New Orleans, Louisiana and ALLEN W. CHEEVER, NIAID, NIH, Bethesda, Maryland.

118. Effect of Schistosoma mansoni on Heart Rate and Oxygen Consumption of Biomphalaria glabrata. CARL S. HACKER, Rice University, Houston, Texas.

119. Ultrastructure of the Cercarienhüllen Reaktion of Schistosoma mansoni. W. M. KEMP, Tulane University, New Orleans, Louisiana.

120. Cercariophagic Activity of Guppy Fish (Lebistes reticulata) Determined with Radioactive Cercariae. WILDA B. KNIGHT, National Communicable Disease Center, San Juan, Puerto Rico, L. S. RITCHIE and J. CHIRIBOGA, Puerto Rico Nuclear Center, Rio Piedras, Puerto Rico.

121. Scanning Electron Microscopy of Schistosome Cercariae, Schistosomules, and CHR Reactions. P. L. MORIEARTY and R. M. LEWERT, University of Chicago, Chicago, Illinois.

122. Egg-shell Histochemistry in Four Species of Digenetic Trematodes. PAUL M. NOLLEN, Western Illinois University, Macomb.

123. Fractionation of Particle-Associated Antigens and Enzymes of Trichinella spiralis by Isoelectrofocusing. DICKSON D. DESPOMMIER, The Rockefeller University, New York, New York.

124. The Use of a Soluble Antigen Fluorescent-Antibody Test to Diagnose Trichinella spiralis in Swine. ROBERT S. ISENSTEIN, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

125. Further Characterization of Haemonchus contortus Exsheathing Fluids. NAIL H. OZEROL and PAUL H. SILVERMAN, University of Illinois, Urbana.

126. Anterior Nervous System of Macracanthorhynchus hirudinaceus. T. T. DUNAGAN and D. M. MILLER, Southern Illinois University, Carbondale.

127. Scanning Electron Microscopy - Observations on a Selected Group of Parasites. P. A. MADDEN and J. M. VETTERLING, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

128. Effect of Temperature on the Feeding Mechanisms of Nymphal Hyalomma aegyptium Ticks. G. K. SWEATMAN, Schools of Medicine and Public Health, American University of Beirut, Lebanon and J. D. GREGSON, Canada Department of Agriculture, Kamloops, British Columbia.

129. Amino Acids in the Egg Shell of Rhipicephalus sanguineus. BENEDICT J. JASKOSKI and VERONICA L. BUTLER, Loyola University, Chicago, Illinois.

Thursday Afternoon - Session 13 - 6 Nov, 1:50 PM
Palladian Room

FINE STRUCTURE OF PARASITES
Chauncey G. Goodchild
Emory University, Atlanta, Georgia, Presiding

130. An Electron Microscope Study of *Entamoeba histolytica* Trophozoites *in vivo*. J. H. MILLER, LSU Medical Center, New Orleans, Louisiana, R. H. GILMAN, University of California Institute for Medical Research, Kuala Lumpur, Malaysia, and V. M. VILLAREJOS, LSU-ICMRT San Jose, Costa Rica.

131. The Fine Structure of Merozoites and Resulting Gametocytes of *Leucocytozoon simondi*. SHERWIN S. DESSER, University of Toronto, Ontario, Canada and JOHN R. BAKER, London School of Hygiene and Tropical Medicine, London, England.

132. Cytochemistry of the Pellicle of *Trypanosoma brucei* and its Role in Cell Shape. K. A. WRIGHT and H. HALES, School of Hygiene, University of Toronto, Canada.

133. Fine Structure of Cephalic Gland Cells of *Cryptocotyle lingua* Cercariae. PAUL L. KRUPA, The City College of New York and ARYA K. BAL and GILLES H. COUSINEAU, Montreal University, Montreal, Canada.

134. Morphology of Post-Embryonic Stages of the Tapeworm *Hymenolepis citelli*. WILLIAM K. COLLIN, University of California, Los Angeles.

135. Rostellar Morphogenesis in *Taenia crassiceps*. P. M. MOUNT, Tulane University, New Orleans, Louisiana.

136. Origin and Possible Utilization of Small Dense Granules in Oocytes of *Ascaris lumbricoides*. W. EUGENE FOOR, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana.

137. Ultrastructure of the Rectal Gland Cells in the Larva of Dirofilaria immitis. HARLEY G. SHEFFIELD and PAUL P. WEINSTEIN, NIAID, NIH, Bethesda, Maryland.

138. Ultrastructure of the Ovejector Diverticulum of Rhigonema infecta (Nematoda: Rhigonematidae). GEORGE S. HAMADA, Tufts University School of Medicine, Boston, Massachusetts.

139. Ultrastructure and Cytochemistry of the Intranuclear Inclusion Bodies of the Intestinal Cells of Obeliscoides cuniculi. M. A. FERNANDO, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

140. Influence of Fertilization on Ovogenesis in Ancylostoma caninum. L. F. LEJAMBRE and J. R. GEORGI, New York State Veterinary College, Cornell University, Ithaca.

141. Surface Ultrastructure in Developing Moniliformis dubius. R. D. WRIGHT and R. D. LUMSDEN, Tulane University, New Orleans, Louisiana.

142. Nemertean Epidermis: A Host-Parasite Interface. JOHN A. OAKS, Tulane University, New Orleans, Louisiana.

Friday Morning - Session 14 - 7 Nov. - 9:00 AM

Diplomat Room

GENERAL - GROWTH OF PROTOZOA AND HELMINTHS IN VITRO

Donald V. Moore
University of Texas, Southwestern Medical School, Dallas, Presiding

143. The Growth and Survival of Amastigotes of Leishmania donovani in Cultures of Peritoneal Macrophages. DONALD W. TWOHY, JAMES R. FORD and VIRGINIA FUNG, Michigan State University, East Lansing.

144. Cinemicrographic Observations on Refractile Body Changes in Sporozoites of Poultry Coccidia in Cell Culture. RONALD FAYER, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

145. Eimeria tenella: Asexual and Sexual Development in Cell Cultures. R. G. STROUT and C. A. OIELLETTE, University of New Hampshire, Durham.

146. Development of Eimeria alabamensis in Cell Cultures. J. ROBERT SAMPSON and DATUS M. HAMMOND, Utah State University, Logan.

147. Development of Eimeria callospermophili in Cell Cultures. CLARENCE A SPEER and DATUS M. HAMMOND, Utah State University, Logan.

148. Temperature Tolerance of Echinostoma revolutum on the Chick Chorioallantois. BERNARD FRIED and DAVID A. FOLEY, Lafayette College, Easton, Pennsylvania.

149. Effects of Some Vitamin Antimetabolites on in vitro Development of Hymenolepis diminuta. LARRY S. ROBERTS and FAITH N. MONG, University of Massachusetts, Amherst.

150. Development in vitro of Hymenolepis microstoma from Cysticercoid to Adult. JAMES S. SEIDEL, University of California, Los Angeles.

151. Environmental Effects on Strongyloides fulleborni in Culture. E. J. BUECHER, E. L. HANSEN, Clinical Pharmacology Research Institute, Berkeley, California, and A. K. BERNTZEN, Portland State University, Portland, Oregon.

152. In vitro Cultivation of Oesophagostomum columbianum in a Simplified Medium with Subsequent Isolation of Parasite Antigens. J. T. MCL. NEILSON, CSIRO McMaster Laboratory, Sydney, Australia.

153. Feasibility of Utilizing *in vitro* Grown Parasitic Nematodes to Detect Anthelmintic Activity. S. E. LELAND, JR., Kansas State University, Manhattan.

154. *In vitro* Cultivation of *Ancylostoma tubaeforme* from Egg to Fourth Stage Larvae and Maintenance of Adults. G. F. SLONKA and S. E. LELAND, JR., Kansas State University, Manhattan.

155. *In vitro* Cultivation of *Cooperia punctata* from Third-stage Larvae to Adults which Produced Eggs that Hatched and Developed to Second-stage Larvae. G. L. ZIMMERMAN and S. E. LELAND, JR., Kansas State University, Manhattan.

Friday Morning - Session 15 - 7 Nov. - 9:00 AM

Tudor Room

CHEMOTHERAPY AND BIOCHEMISTRY - PROTOZOA AND HELMINTHS

Paul E. Thompson
University of Georgia, Athens, Presiding

156. The FDA Looks at Veterinary Anthelmintics. JOHN B. POOLE and JAMES H. MARK, Bureau of Veterinary Medicine, FDA, Washington, D. C.

157. Effects of Two New Triaminoquinazolines on Malaria in Animals. PAUL E. THOMPSON, ANITA BAYLES and BRONISLAWA OLSZEWSKI, Parke, Davis and Company, Ann Arbor, Michigan.

158. Effect of *Catalpa bignonioides* on the Parasites of Poultry. PAUL D. HARWOOD, Hess and Clark Division of Richardson-Merrill Inc., Ashland, Ohio.

159. New Agent for Treatment of Fascioliasis. W. C. CAMPBELL, D. A. OSTLIND, R. F. RIEK and J. J. YAKSTIS, Merck Institute for Therapeutic Research, Rahway, New Jersey.

160. Efficacy of Parbendazole Against Gastrointestinal Nematodes in Angora Goats. V. J. THEODORIDES, G. C. SCOTT and MARVIN LADERMAN, Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

161. Anthelmintic Efficacy of Maretin in Two Controlled Tests with Calves. D. D. COX and M. T. MULLEE, Chemagro Corporation, Kansas City, Missouri.

162. Efficacy of Baymix Against Gastrointestinal Nematodes in Two Controlled Tests with Calves. M. T. MULLEE and D. D. COX, Chemagro Corporation, Kansas City, Missouri.

163. Efficacy of Thiabendazole Against Two Ovine Isolates of Haemonchus contortus. M. L. COLGLAZIER, K. C. KATES, and F. D. ENZIE, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

164. Efficacy of Thiabendazole, l-Tetramisole, and Parbendazole Against Natural Infections of Helminths of Sheep. K. C. KATES, M. L. COLGLAZIER, F. D. ENZIE, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland and I. L. LINDAHL and G. SAMUELSON, JR., AH, ARS, USDA, Beltsville, Maryland.

165. New Agent for Treatment of Haemonchosis. J. R. EGERTON and W. C. CAMPBELL, Merck Institute for Therapeutic Research, Rahway, New Jersey.

166. The Effect of Levo-Tetramisole on Experimentally Induced Infections of Trichostrongylus axei and Ostertagia ostertagi in Calves. G. H. ROHRBACHER, J. EMRO and E. WALETZKY, American Cyanamid Company, Princeton, New Jersey.

167. Ultrastructural Changes Occurring in Dirofilaria immitis after Treatment with Caparsolate Sodium. CHIN-CHIU LEE, King's College, Wilkes-Barre, Pennsylvania.

168. Movement of Long Chain Fatty Acids Across the Mid-Gut of Ascaris lumbricoides suum. CALVIN G. BEAMES, JR., and GARY A. KING, Oklahoma State University, Stillwater.

169. Localization and Distribution of DNA in Nuclei of Developing Ascaris lumbricoides var. suum. S. R. SYLK, School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

170. Mouse Peritoneal Monocyte Reactions to *Ascaris suum* Larvae. E. L. JESKA, Veterinary Medical Research Institute, Iowa State University, Ames.

Friday Afternoon - Session 16 - 7 Nov. - 1:30 PM
Diplomat Room

IMMUNOLOGY AND SEROLOGY - PROTOZOA, HELMINTHS AND ARTHROPODA
Irving G. Kagan
National Communicable Disease Center, Atlanta, Georgia,
Presiding

171. Lysozyme Action in the Symbiotic and Symbiont-free Cockroach. D. R. A. WHARTON, U. S. Army Natick Laboratories, Natick, Massachusetts.

172. A Common Mechanism of Immunity for Intracellular Infections. ALICE YURCHISION and JACK S. REMINGTON, Palo Alto Medical Research Foundation and Stanford University School of Medicine, Palo Alto, California.

173. Immunoelectrophoretic Comparisons of Some *Entamoeba* Antigens. M. N. LUNDE and L. S. DIAMOND, NAID, NIH, Bethesda, Maryland.

174. Protective Effect of Noninfective Forms of *Trypanosoma cruzi* in Culture-Initiated Infections. ROBERT G. YAEGER and ELSA L. WINSOR, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana.

175. Action of the Thymus Extract in Experimental Chagas Disease. J. OTTILIO L. MACHADO, University Federal Fluminense, Rio de Janeiro, Brazil.

176. Immunologic Selection for a Mutant or Relapse Strain of *Plasmodium lophurae* in White Pekin Ducklings. R. M. CORWIN and H. W. COX, Michigan State University, East Lansing.

177. Immunization of Mice with Parasite Antigens of *Plasmodium berghei* and *Babesia rodhaini*. KYLE H. SIBINOVIC, Howard University, Washington, D. C.

178. Preliminary Studies on the Use of Ferritin-Conjugated Antibodies to *Plasmodium berghei*. VIRGINIA A. A. KILLBY and PAUL H. SILVERMAN, University of Illinois, Urbana.

179. Anti-lymphocyte Serum Effects on *Plasmodium berghei* Infection in Rats. DAN T. SPIRA and PAUL H. SILVERMAN, University of Illinois, Urbana.

180. Effect of Immunosuppression on Recovery from Primary *P. berghei*. LEE R. BARKER and KENDALL G. POWERS, NAID, NIH, Bethesda, Maryland.

181. *Plasmodium vivax* Infections in *Ateles* Monkeys. J. A. PORTER, Veterans Administration, Edward Hines, Jr. Hospital, Hines, Illinois and M. D. YOUNG, Gorgas Memorial Laboratory, Panama, Republic of Panama.

182. Serological Relationships of *Plasmodium* Species in Malaria IFA Tests. A. J. SULZER and MARIANNA WILSON, National Communicable Disease Center, Atlanta, Georgia.

183. Mosquitoes Feeding on Engorged Mosquitoes. A. B. WEATHERSBY, HYONG-SUN AH and J. W. MCCALL, University of Georgia, Athens.

184. Immunologic Studies of Larval *Echinostoma lindoense* in the Snail Host. DONALD HEYNEMAN, Hooper Foundation, and W. PAGE FAULK, University of California Medical Center, San Francisco.

185. Immunological Studies on the Canine Hookworm, *Ancylostoma caninum*. J. C. WILLIAMS, Louisiana State University Medical Center, New Orleans.

Friday Afternoon - Session 17 - 7 Nov. - 1:30 PM
Palladian Room

BIOCHEMISTRY AND GENERAL -
PROTOZOA, HELMINTHS AND ARTHROPODS
Robert B. Short
Florida State University, Tallahassee, Presiding

186. Some Biochemical Aspects of the Systematics of *Proterometra* spp. (Trematoda:Azygiidae). WILLIAM B. LUSHBAUGH, LSU School of Medicine, New Orleans, Louisiana, and MARLOWE G. ANDERSON, New Mexico State University, Las Cruces.

187. Changes in the Free Amino Acid Concentration of Bile During Infection by *Fasciola hepatica* L. HADAR ISSEROFF and CLARK P. READ, Rice University, Houston, Texas.

188. Glucose Uptake and Glycogen Synthesis in *Schistosoma mansoni*. S. H. ROGERS, School of Medicine, and E. BUEDING, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

189. Sequential Appearance of 3 Major Perivitelline-Fluid Macromolecules During Development of *Ascaris lumbricoides* var. *suum*. D. B. SMITH, University of Massachusetts, Amherst.

190. Ultrastructure of the Conoid Apparatus of Dormant and Activated Sporozoites of *Eimeria*. JOHN M. VETTERLING and P. A. MADDEN, Beltsville Parasitological Laboratory, ADP, ARS, USDA, Beltsville, Maryland.

191. Habitat Segregation in Blood Flukes of Rockfishes from the Pacific Coast. JOHN C. HOLMES, University of Alberta, Edmonton, Alberta, Canada.

192. Pre- and Postimpoundment Surveys of Helminth and Crustacean Parasites of Black Basses. DAVID A. BECKER, University of Arkansas, Fayetteville.

193. Preliminary Report on Some Trematodes of Fishes from South America. ERNEST J. HUGGHINS, South Dakota State University, Brookings.

194. Trematodes of the Genus Renicola Cohn, 1904 (Trematoda: Digenea:Renicolidae). ELON E. BYRD, University of Georgia, Athens, RICHARD W. HEARD and FORREST E. KELLOGG, School of Veterinary Medicine, University of Georgia, Athens.

195. A New Record of Turtle Cestode in Louisiana. ALEXANDER D. ACHOLONU, Southern University, Baton Rouge, Louisiana.

196. Wyominia tetoni: Its Host, Distribution, and Other Relationships. REX W. ALLEN, Animal Disease and Parasite Research Division, ARS, USDA, Las Cruces, New Mexico.

197. Ecology of the Acanthocephalan, Acanthocephalus dirus, in the Intermediate Host, Asellus intermedius (Crustacea: Isopoda). ARTHUR J. SEIDENBERG, Virginia Commonwealth University, Richmond.

198. Ectoparasite-Host Relationships among Pichinde (Colombia) Small Mammals. HAROLD TRAPIDO, The Rockefeller Foundation, Facultad de Medicina, Universidad del Valle, Cali, Colombia.

199. Amblyomma (Acarina:Ixodidae) on White-tailed Deer from South Texas. W. M. SAMUEL, University of Wisconsin, Madison.

1

Intestinal starch digestion and absorption in rats infected with Hymenolepis diminuta, ROBERT W. MEAD* and LARRY S. ROBERTS

The digestion and absorption of carbohydrate after a starch meal was studied by a method modified from Dahlquist and Thomson (1963, Acta Physiol. Scand. 59:111) in rats infected with Hymenolepis diminuta. Carbohydrate disappeared at a significantly higher rate from the small intestines of rats infected with the cestode than from the intestines of control rats. The effect was observed 1.5 to 2.5 hr following the starch meal, i.e., while the starch was located in the middle and posterior thirds of the small intestine, and it probably reflects the consumption of a portion of the digesta by the tapeworms. The amount of free glucose recovered from the intestines of infected and control rats was similar at all intervals tested after the starch meal except at 2 hr. At that time there was more free glucose in the posterior third of the gut of infected rats. This observation is not explained as yet. (Supported in part by NIH grants AI-06153 and AI-40034.)

2

Organism weight and tissue lipids of rat host and Hymenolepis diminuta following dietary lipid alteration. CHAUNCEY G. GOODCHILD and ARDIS L. CRAMER*

Forty weanling male Wistar strain rats were divided into 5 groups with the closest possible similarity of weight. Five different diets supplied identical casein, mineral, and vitamin amounts and equal caloric value, using 5 admixtures of cornstarch and cottonseed oil (9:4 w/w compensation ratio). After 7 weeks of diet each group was divided into control and infected subgroups. A group of 25 young adult rats was started on the diets and simultaneously infected. A 5 worm infection with Hymenolepis diminuta produced, at 14 days, differences in percentage of microsoxhlet chloroform-methanol extract of intestinal wall and skin when a diet containing 9 percent oil was provided. The diets produced differences in worm and host weight and in amount extracted from the tissues. Nine correlations among extract percentage of host tissues and between worm and host tissues were limited by length or type of diet; 3 were not. Extract percentage of tapeworms was raised only when the host was simultaneously infected and confronted with a diet rich in oil. Long term oil-rich or adequate diet was associated with tapeworms of lighter weight. Extract of tapeworms contained bile acid and pigment and yielded fatty acids whether or not they had been provided in the diet. (Supported by NSF Cooperative Graduate Fellowship 1963-1966; equipment provided, in part, from Training Grant 3T01 AI00037 of the National Institutes of Health)

3

Changes in the Amino Acid Pool of Hymenolepis diminuta (Cestoda) Associated with Induced Imbalance of Exogenous Amino Acids. LESLIE H. CHAPPELL* and CLARK P. READ.

The effects of an excess of a single amino acid on the free amino acid pool of 10-day old H. diminuta were investigated. Lysine, proline and phenylalanine, at a concentration of 2mM, were included separately in incubation media (KRT) containing 10 tapeworms. Worms were removed after various times and either killed and extracted in ethanol or incubated further in a "normal" amino acid mixture, for varying times, prior to ethanol extraction. Analogous experiments were carried out in vivo using rats fed per os with high doses of a chosen amino acid. The levels of the constituent amino acids in the tapeworm pool were determined with an amino acid analyser. Accumulation of the imbalanced amino acid was accompanied by efflux of certain other pool acids; during short time periods little regulation of the worm pool was observed.

4

Tegumentary Phosphatases of Hymenolepis diminuta. SUE CARLISLE DIKE* and CLARK P. READ

Surface phosphatase activity in H. diminuta has been characterized using non-permeating fructose esters as substrates. Enzymatic hydrolysis of these compounds is optimal at pH 7.0-7.3. This hydrolysis is not inhibited by sodium fluoride. It is inhibited, however, by phlorizin, ammonium molybdate, adenosine triphosphate, adenosine monophosphate, and a variety of phosphorylated sugars.

In contrast, when p-nitrophenyl phosphate is used as a substrate, no inhibition occurs in the presence of phlorizin or phosphorylated sugars. Ultrastructural localization of enzymatic activity using fructose-1-phosphate or p-nitrophenyl phosphate as substrates shows both of these compounds to be hydrolyzed on or near the surface; however, the biochemical data suggest that at least two separate enzymatic entities capable of the hydrolysis of phosphate esters are operative in this tapeworm. (Supported in part by NIH Grants 5TI AI0106 and AI01384.)

5

Effects of Thymine and Thymine Analogs on the in vitro Uptake of Uracil-2-C^{14} by Hymenolepis diminuta. AUSTIN J. MACINNIS and ROBERT K. RIDLEY.*

Uracil at 0.1mM is taken up by Hymenolepis diminuta at a rate of 1.88±.04μM/g dry wt/hr, confirming the results of MacInnis, et al (J. Parasit. 51:260-267, 1965). Thymine (5-methyluracil) at concentrations of 0.5, 2, 5, and 10 mM stimulated the uptake of 0.1mM uracil by 23%, 72%, 106%, and 88% respectively. A 27% inhibition of uracil uptake was obtained by incubating with 2mM 6-methyluracil: 2mM 5-aminouracil increased the amount of uracil uptake by 31%; 2mM 5-carboxyuracil, 2mM 5-

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hydroxymethyluracil, and 2mM cytosine (2-oxy-4 amino pyrimidine) had no observed effect on the rate of uracil uptake. Preincubating in 2mM thymine for 30 minutes resulted in a 113% stimulation of uracil uptake. From these data it was concluded that the presence of the methyl group on the five position of the pyrimidine ring provided the molecular configuration that gave the most stimulation of uracil uptake. Substituting a carboxyl or hydroxymethyl for the methyl group on the five position gave a configuration that did not cause stimulation or inhibition of uptake. When the methyl group was on the six position, the molecule caused inhibition of uracil uptake. Potentiation of transport of pyrimidines by thymine may provide a mechanism for increasing permeability to analogs that may effectively inhibit nucleic acid synthesis, but are not readily accumulated by the parasite.

This research was supported by NIH AI00070, the University of California Research Grant No. 2280, and NSF 5167.

6

Characterization of Hymenolepis diminuta DNA. CLINT E. CARTER*, AUSTIN J. MACINNIS and ROBERT K. RIDLEY.

DNA was extracted from H. diminuta using a modification of a technique described by Searcy (B. and B. Acta 1968). The G-C-content calculated from buoyant density, melting temperature, and DNA bromination data was 38%. Investigations made on this DNA using cesium chloride density gradient centrifugation revealed the presence of one main band and two satellite bands. Observations were made on purified DNA from the main band and satellite bands by electron microscopy. The three bands appear to correspond to nuclear, mitochondrial, and an as yet unidentified satellite DNA.

Supported by grants NIH AI00070, NSF 5167, and University Research 2280.

7

Mitotic activity and thymidine incorporation in the germinative region of developing Hymenolepis diminuta, R. I. BOLLA* and L. S. ROBERTS

The area immediately posterior to the scolex is apparently responsible for the generation of new proglottids in most cyclophyllideans. Cell multiplication in this germinative region has been studied in Hymenolepis diminuta during the prepatent period in the definitive host. Worms were recovered from rats from 2 to 14 days postinfection and treated with colchicine. After staining by the Feulgen reaction, mitotic indices in the germinative areas were determined. The mitotic index was approximately 5 times greater in 2-4 day old worms than in worms recovered 10-14 days postinfection. Other worms recovered at similar time periods after infection were subjected to pulse chase incubation in tritiated thymidine, and autoradiographs of the germinative regions were prepared. The labeling index was about 5 times higher at 2 days than at 12 days postinfection. Although the cells which incorporated label were distributed randomly through the subcuticular and parenchymal regions in 2 and 4 day old worms, incorporation was limited to a band just internal to the parenchymal muscle zone from 6 days onward. Neither subcuticular cells nor cells in the central parenchymal region incorporated significant amounts of thymidine after 4 days. This localization of labeled cells paralleled the morphological localization of mitotically active cells.

(Supported in part by NIH grants AI-06153 and 5 TOI-AI-226.)

7

8

Measurements of the Genetic Basis of Parasitic Reduction, DENNIS G. SEARCY* and AUSTIN J. MACINNIS

The size of the genome of the parasitic plant Cuscuta californica was compared to the sizes of the genomes of several closely related autotrophic plants. Data obtained by reciprocal DNA hybridizations in vitro and by measurements of the kinetics of DNA renaturation are in agreement, and indicate that the parasite Cuscuta has a genome about 50% the size of the autotrophic plants Ipomoea and Convolvulus, and about 90% the size of the autotrophic plant Dichondra. By comparing the rate of DNA renaturation to that of E. coli DNA, it is possible to determine the absolute size of the genome. Excluding the repetitive sequences of the DNA, the approximate molecular weights of the genomes are: Cuscuta = 3.3×10^{10}; Ipomoea = 6.9×10^{10}; Convolvulus = 8.0×10^{10}; and Dichondra = 4.7×10^{10}.

While these data indicate that some parasites can have a reduced genome, preliminary data indicate that the cestode Hymenolepis has a genome about three times larger than the turbellarian Dugesia.

Supported by PHS Training Grant AI00070 and NSF Grant GB 5167.

9

Effects of Ultraviolet Light on Hymenolepis microstoma. J. H. BUTLER and F. H. WHITTAKER*

Cysticercoids of Hymenolepis microstoma were irradiated with ultraviolet light of 200μW/cm^2 for 24 and 36 minutes. These cysticercoids, as well as a control group, were fed to albino mice. Worms were recovered after 13 days. Effects on infectivity and growth, as well as qualitative and quantitative effects on morphology were noted. The abnormalities were recorded under four main categories; testes development, cirrus pouch formation, sterility and segmentation. An analysis of variance test revealed that there is a significant difference at the 0.01 level in infectivity and growth between experimental and control worms. A Chi-square test showed that the difference in number of abnormalities between control and experimental worms was significant at $P < 0.01$. Therefore, it seems that ultraviolet

radiation has a damaging or lethal effect on cysticercoids of H. microstoma. The lethal effect is most likely due to absorption of the radiation by nucleic acids, and the abnormalities may possibly be explained by absorption of the radiation by enzymes and other proteinaceous materials.

10

Gamma Radiation-Induced Effects on Genetic Fitness of Hymenolepis nana,

PAUL H. RODRIGUEZ* and J. W. CRENSHAW

The genetic effects of low dosage gamma radiation on H. nana after it had become established in a beetle vector, Tribolium confusum, was examined. Adult male and female T. confusum were infected with the parasite, then irradiated with 500 r, 1,000 r, and 2,000 r nine days after infection. Subsequently, the cysticercoids were recovered from the beetles, transferred to mice, and the adult cestodes themselves recovered. After second transfer to beetles, second generation cysticercoids were obtained and inoculated into mice again for recovery of second generation adult cestodes. Different groups of non-irradiated, inbred strains of T. confusum and DBA/2J mice were employed to maintain the various generations of the parasite. Gamma irradiation with 500 r seems to induce an increased fitness, as measured by viability, of the irradiated parasite. This trait in turn appears to be transmitted to second cysticercoids and adult cestodes alike. Higher doses of gamma irradiation, however, appear to decrease fitness.

(Supported by NIH Fellowship 5-F01-GM30282-03 to P.H.R.)

11

Cytochemical observations on the mitochondrial oxidation of 3-3' diaminobenzidine (DAB)

R.D. LUMSDEN*, J.A. OAKS and R.R. MILLS

Oxidation of DAB results in formation of an insoluble, osmiophilic polymer. This has been used in the ultrastructural localization of peroxidase activity (Graham and Karnovsky, 1966, J.H.C. 14: 291). Recent reports of a peroxidase in cestode and trematode mitochondria stem from results obtained with this technique. Further characterization of the cytochemically apparent peroxidatic oxidation of DAB by tapeworm mitochondria is reported herein and compared with the same reaction observed in mammalian mitochondria and microbodies. Activities in all systems studied are similar in their heat lability and inhibition by azide and cyanide. However, oxidation of DAB by rat and cestode mitochondria, unlike that by hepatic microbodies, is insensitive to 3-amino, 1,2,4 triazole. Further, at sufficiently high concentrations of the oxidant, heat labile, cyanide and azide sensitive mitochondrial oxidation of DAB was observed in the absence of H_2O_2. Infrared spectra of the reaction product formed on incubation of $H_2O_2$and DAB with purified horseradish peroxidase, heart muscle and cestode homogenates were found to be identical in the -NH stretching region above 3,000 cm^{-1}. It thus seems possible that the peroxidatic activity of helminth mitochondria is not due to a true peroxidase but reflects an ancillary function of some other hemoprotein(s), such as the cytochrome -cytochrome oxidase system. [Supported by grants from the NIH (AI 08673, 5TI GM 669), NSF (GB 7276) and Career Development Award K04-AI 23449 (to RDL) from the USPHS, NIAID.]

12

α-Glycerophosphate Oxidase of Taenia taeniaeformis

EUGENE C. WEINBACH* and THEODOR VON BRAND
National Institutes of Health
Bethesda, Maryland

A polarographic study of mitochondria isolated from larval or adult Taenia taeniaeformis disclosed that the substrate most readily oxidized was α-glycerophosphate. The product of the oxidase reaction was dihydroxyacetone phosphate. Hydrogen peroxide was not detected, but the presence of an active catalase may have prevented its identification. Oxidation of α-glycerophosphate was not mediated by NAD, but was stimulated by Vitamin K_3. The oxidase was inhibited by dicoumarol, cinnamate, and metal chelating agents. Antimycin and cyanide were only partially inhibitory. There was little evidence of a classical cytochrome system participating in terminal electron transport, but non-heme iron was functional in the oxidase reaction. The cytoplasm of the parasite contained an extramitochondrial, NAD-linked, α-glycerophosphate dehydrogenase. The presence of these essential enzymes of the α-glycerophosphate cycle in T. taeniaeformis may provide an important mechanism for the oxidation of NADH generated during glycolysis.

13

Biosynthesis de novo of purines and pyrimidines in Mesocestoides (Cestoda),

ROBERT L. HEATH and JOSEPH L. HART*.

Tetrathyridia, maintained in vitro in a synthetic medium containing preformed purines and pyrimidines fail to synthesize these compounds de novo. However, if the organisms are placed in a medium lacking in preformed purines and pyrimidines, incorporation of ^{14}C-labeled orotic acid into nucleic acid pyrimidines is observed, as indicated both by isolation, hydrolysis, and chromatography of the nucleic acids and by autoradiography of histological specimens before and after treatment with nucleases. The results are interpreted as indicating derepression of the genes controlling pyrimidine biosynthesis under these conditions. Indirect evidence is presented that the organisms are also capable of de novo synthesis of purines in the absence of an external source of preformed purines. (Supported in part by NIH Grant 5-T01 AI-00249).

The Action of Bile Salts on Hymenolepidid Cysticercoid Hatching. RICHARD D. TKACHUCK

The action of bile salts on the hatching rate of three species of hymenolepidid tapeworm cysticercoids was examined using the method of Rothman, 1959 (Exp. Parasit. 8:336). Three bile salts, taurocholate, taurodeoxycholate, and taurochenodeoxycholate, were tested. Rates of hatching could be directly related to the ability of each bile salt to solubilize lipid. Cysticercoids activated in taurocholate took up eight times more tritiated water than non-activated controls which were incubated without taurocholate. Supported by NIH AI00070, NSF 5167, and University Research 2280 grants.

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System for Determining Percent Survival of Entamoeba histolytica cysts in vitro, RICHARD STRINGER*

Excysted Entamoeba histolytica leave behind a translucent cyst wall which can easily be distinguished from the full unexcysted form utilizing high dry magnification. Based on the assumption that an empty cyst represents an amoeba which was viable enough to crawl out of its cyst while a full cyst represents an inhibited or dead cyst, a system has been developed for determining the percent of viable cysts in a sample. A cyst suspension containing 80,000 cysts which have been allowed to excyst are placed on a slide and covered with a vasoline-edged cover slip. Five hundred full and empty cysts are counted. The percent of excysted cysts in a sample is determined by the formula $\frac{\text{No. empty cysts counted}}{\text{No. full and empty cysts counted}} \times 100$. When the percent of cysts surviving is corrected for controls the following formula is used: $\frac{\text{Percent of cysts surviving in test}}{\text{Percent of cysts surviving in control}} \times 100$. The potential of this system for studying factors governing excystation and for evaluating potency of cysticidal compounds for cysts of Entamoeba sps., and other protozoa, is great. It has been used to determine the length of time required for a percent of viable strain 200:NIH cultured Entamoeba histolytica cysts to excyst. At 37°C in a culture containing 0.4 ml of a cyst suspension, 2.6 ml Diamond's TTP media, 2364 units of penicillin, and 1575 units of streptomycin excystation began after 15 minutes. Fifty percent of the viable cysts had excysted in 5 hours and 100% had excysted in 10 hours. The system offers an improvement over previous systems which were only sensitive enough to detect 100% mortality, or some cyst survival. Percent survival can be determined by the new system.

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Development of Elongate Gametocytes of Leucocytozoon simondi in Erythroblasts in the Bone Marrow of White Pekin Ducklings, A. L. AGER* and R. M. CORWIN

Late stages of developing elongate gametocytes of Leucocytozoon simondi have been described in the peripheral circulation and previously it was suggested that erythrocyte precursors might serve as host cells (Desser, 1967). In bone marrow of White Pekin ducklings infected intraperitoneally with sporozites which had been stored in liquid nitrogen at -196°C, we have observed a complete sequential development of the elongate form in the proerythroblast.

Earliest stages of developing elongate gametocytes were noted 10 days after infection and appeared as small round forms in the proerythroblast. The staining properties of the young gametocyte appears almost identical to that of the host cell cytoplasm except for the red nucleus of the parasite which may explain why this stage has not been described before. Subsequent stages show the elongation of the parasite with a concurrent flattening of the host cell nucleus and masking of the host cell cytoplasm except for the lateral horns. This transition takes place within a 24 hour period with elongate gametocytes appearing in the peripheral circulation as mature or nearly mature forms. Thus the bone marrow appears to be a primary site for development of elongate gametocytes with the erythroblast serving as a host cell.

Round gametocytes were seen earlier in monocytes or in immature lymphocytes in the peripheral circulation but not in the bone marrow. Lymphoid organs have not yet been studied.

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Plasmodium tenue Laveran and Marullaz 1914. R. D. MANWELL

The original description of this species was very brief and poorly illustrated, No one seems to have studied it since. Due to apparent similarity to P. vaughani, very common in the American robin (Turdus migratorius, a thrush) and because the host in which it was found (Liothrix luteus, a babbler) is often called the Pekin robin, P. tenue has been generally thought to be a synonym of P. vaughani. However what is undoubtedly P. tenue has recently been found in several specimens of Liothrix luteus and there seems little doubt that the two species of Plasmodium are distinct, although there are similarities. P. tenue lacks the refractile granules typical of P. vaughani and is not transmissible to canaries (vaughani is). Both species usually produce 4 merozoites per segmenter (but tenue may give rise to 6, and vaughani to 8). Gametocytes of both are elongate and do not curl about the red cell nucleus; neither they nor the asexual stages displace it. There is reason to think both species of malaria parasite are more or less specific for their respective host species. Vectors and exoerythrocytic stages of both are unknown. Both belong to the subgenus Novyella. Aided by NIH Grant AI 05182-07.

ABSTRACTS

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Attachment Organ Development of Cotylurus variegatus in Larus delawarensis, M. E. HAIGHT* and D. N. JENSEN

Maturing C. variegatus were obtained from L. delawarensis which had been infected, per os, with encysted metacercariae previously removed from Perca flavescens. Prior to attachment the body and organ sizes of juvenile parasites decreased approximately 30%. With the onset of attachment and feeding, which occurs approximately 18 hours post infection, rapid growth begins and at the end of 3 days body sizes have increased 110%, oral sucker 60%, ventral sucker 100%, pseudosuckers 130% and adhesive organ 215% as compared to the original sizes. Attachment is initiated when the pseudosuckers in the protruded cup-shape position adhere to a portion of the gull's intestinal tissue. Subsequently the oral sucker by muscular sucking action draws in portions of tissue. The pseudosuckers become invaginated deep cavities containing adherent host tissue. No attachment function could be observed for the ventral sucker. As the adhesive organ lobes develop and surround the host tissue, growth of the ventral suctorial lip produces the characteristic cup-shaped forebody. The areas of contact between portions of adhesive organ lobes, pseudosuckers, and host tissue result in the formation of an intimate and permanent host-parasite interface which histologically resembles a placenta. Studies of this interface using electron microscopic techniques are in progress.

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Host and Organ Specificity of the Metacercarial Cysts of the Genus Ascocotyle Looss, 1899, P.C. STEIN* and R.D. LUMSDEN

The genus Ascocotyle in the family Heterophyidae presently includes 28 species. Brackish water fish belonging to the families Cyprinodontidae, Poeciliidae and Mugilidae have been found to be the usual second intermediate hosts. Fish collected in Florida and Louisiana were examined for the presence of the metacercarial cysts. Measurements of the cysts indicate that there is considerable variability between the species. Cysts were observed to have a characteristic shape and size regardless of the organ infected or the fish host. Light and electron microscopy of the metacercarial cysts demonstrates the possible role of the host and parasite in the production of the cyst wall. In addition, a layer of host fibroblasts occasionally containing melanocytes is often observed to be closely applied to the outer cyst wall. (Supported by grants from NIH (AI-08673), NSF (GB-7276) and Career Development Award K04-AI23449 (to RDL) from the USPHS (NIAID)).

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Intraspecific Variation of Quinqueserialis quinqueserialis in Rodent Hosts. JOHN M. KINSELLA*

The trematode Quinqueserialis quinqueserialis was successfully established in the laboratory in 18 rodent hosts. Sixteen of these have not been reported previously as natural or experimental hosts. The growth rate of the fluke was studied in three hosts: Ondatra zibethica, Microtus pennsylvanicus, and Microtus montanus. Growth was most accelerated in M. montanus (prepatent period 15 days), slower in M. pennsylvanicus (prepatent period 18 days), and slowest in O. zibethica (prepatent period 28 days). In all three hosts growth was shown to continue considerably past the time of sexual maturity. A statistical analysis of this continuing growth showed it basically to be an increase in length, width, and possibly volume. Organ systems appeared to attain stability in size after varying periods of time. Analysis of variation of trematodes from various species of hosts demonstrated a definite host effect on morphology. Specimens from voles of the genus Microtus were consistently larger than natural or experimental specimens from muskrats.
(Supported in part by an NIH predoctoral fellowship).

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Basic Hook Musculature and Movements of Invasive Oncospheres from Hymenolepis diminuta. ROBERT E. OGREN*

The fundamental plan for hook musculature was investigated and used to explain hook movements. The method involved the preparation and study of living and stained whole mounts by light and phase contrast microscopy. Results: A basic pattern of 5 fiber systems existed for each hook:1) Protractors; 2) Abductors; 3) Adductors; 4) Levators; 5) Retractors. Basically similar movements were shown by lateral and medial hooks,but in different planes: Protraction forward beyond body, abduction in an arc away from the main axis and forward-lifting of shank, followed by holding. In retraction hooks were pulled back by adductors toward mid line and retracted into body nearly parallel. The lateral hooks executed a rowing motion, while the medial hooks clawed toward the substrate. The basic scheme made comparison possible and appeared to be a functional model. Correlated hook and body movements occurred in the following sequence: 1) Lateral hook protraction; 2) Anterior enlargement and mid-body constriction;3) Medial hook protraction; 4) Elongation and holding; 5) Retraction;6) Posterior enlargement to spherical form.
(Supported by NSF Grants G12905,GB1480,GB4328).

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Morphology and Histochemistry of Genital Cone of Cooperia punctata and Comparison of Seven Cooperia Species. FRANK STRINGFELLOW

Cuticular and sclerotized components in the genital cones of 156 male Cooperia punctata were studied

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in whole mount and sectioned material prepared by special staining techniques. The structure, function, and histology of the genital cone were determined. Histochemical tests indicated cuticular areas are primarily collagenous-like whereas sclerotized areas are scleroprotein-like material. The genital cone has at least three functions. 1. site for muscle attachment 2. guide for extrusion and retraction of the spicules 3. skeletal support for the dorsal and ventral cloacal walls. Genital cones of 7 species of Cooperia were compared. Genital cones of C. punctata and C. spatulata are identical. Conformation of the sclerotized bars of the distal complex distinguish C. curticei from the two previously mentioned species. Genital cones of C. oncophora and C. bisonis are identical; a sclerotized protuberance, no baso-dorsal plate connection, a sclerotized sheet between the cloacal plate and dorsal plates, a prominent ventro-dorsal plate that fuses medially as a sheet over the dorsal part of the genital cone are diagnostic. C. surnabada has a baso-dorsal plate connection and a cuticular protuberance. C. pectinata differs from all other groups in the conformation of the distal complex, sclerotized projections from the cloacal plate, dorso-dorsal plate junction, small genital papilla, cuticular protuberances from the basal apparatus, and an arched cloacal plate.

23

"Pearl-like nodules" in the macaque monkeys.

M. M. WONG* and H. D. CONRAD

Small "pearl-like nodules" were observed, and reported as such, in approximately 8% of Macaca irus monkeys by Honjo and co-workers in 1963. Observations made in this laboratory during the past 2 years indicated that small nodules of similar appearance could be found in 5 different asian macaques: M. mulatta, M. irus, M. nemestrina, M. speciosa and M. radiata. Closer examinations of these nodules revealed that the etiologic agents may be anyone of the following: (1) larval cestode, either Sparganum or Tetrathyridium; (2) larval nematode belonging to a species of Physaloptera, Streptopharagus, Dispharynx (Acuariidae) or one of the ascarid type; (3) an Acanthocephalan larva and (4) a Pentastomid nymph. Further identifications of some of these were made possible by feeding experiments. However, evidence of host's tissue reaction suggests that these larval parasites are probably of aberrant forms in these monkeys.

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A Comparison of Haematopinus eurysternus from New Mexico and H. quadripertusus from Puerto Rico, W. P. MELENEY

Most modern textbooks on veterinary parasitology treat Haematopinus quadripertusus, the tail or switch louse of cattle, as a distinct and separate species from H. eurysternus, the short-nosed or big gray cattle louse. However, earlier works, on which many veterinarians still depend, and the definitive work, "The Sucking Lice" (Ferris, G. F., Mem. Pacific Coast Entomol. Soc., 1, Oct., 1951: 88-90), either do not mention it or deny its status as a distinct species. Even some of the most recent textbooks neglect it altogether or fail to locate it on cattle in the Western Hemisphere.

In the C.D.C. publication, "Pictorial Keys to Arthropods, Reptiles, Birds and Mammals of Public Health Significance" (U. S. Dept. H.E.W., Public Health Service, Atlanta, Georgia, 1966: 72-73), Stojanovich and Pratt use the outline of the sternal plate of the adults and the number of setae on the genital plate of the adult males as differentiating criteria.

Populations of H. eurysternus from cattle in New Mexico and of H. quadripertusus from cattle in Puerto Rico are compared in relation to the length of the adults, the shape of the adult sternal plate, and the number of setae on the genital plate of the adult males.

It is concluded that the two species are distinct and should be so recognized and classified, but that there is considerable variation in these differentiating characters between the sexes and between individuals of both species.

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Preliminary Taxonomic Value of Antennal Sensilla of the Anoplura.

FREDERICK H. MILLER*

Studies in our laboratories by means of scanning electron microscopy have provided new contributions to the taxonomy of Anoplura (Miller 1969). These studies have revealed that the peg organs of the sensilla coeloconica or sensilla basiconica of the fourth and fifth antennal segments vary in size and in number of protrusions from their apex. There is also a difference in size of the openings of the sensilla coeloconica within a genus. The demonstration of pore organs and other antennal structures may also be of taxonomic significance. These studies have continued and the results of more recent investigations together with illustrations will be presented.

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Lesions in Capra hircus due to Demodex caprae,

W. B. NUTTING* and R. R. LEBEL.

Serial paraffin sections (various stains) of goat skin infested with D. caprae, including all stages of lesion development and decline, were studied using light and phase microscopy. Demodex caprae invades the pilosebaceous system in the adult stage. Adults feed and reproduce thus setting up the following host reactions: (1) marked hyperplasia of follicular epithelium, (2) increase in surrounding vascular network, (3) loss of hair, and (4) closure of pilosebaceous orifice. Rupture

of the mature papulous lesion results in (a) disgorgement of some mites, (b) keratin investment and sloughing of other mites, (c) giant cell phagocytosis of mites not removed by a. or b., and (d) healing of the wound with only moderate scar tissue formation.

Bacteria as secondary invaders after papules rupture are responsible for suppurating lesions. In these keratin investment is retarded, a more extensive inflammatory reaction occurs, the mites are phagocytosed by giant cells, and the healing process is of much longer duration with the formation of extensive amounts of scar tissue.

(Supported in part by NSF Grant GB-3516.)

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Demodex caprae - Structural Features of Use in Demodicid Systematics, C. E. DESCH* and W. B. NUTTING.

All life cycle stages of D. caprae were studied as whole mounts and serial paraffin sections (various stains) using light and phase microscopy. Larval, nymphal and adult palps, palpal setae, chelicerae, pharyngeal bulbs, and podosomal appendages are detailed in stage sequence and (re) named in light of current views on acarine homologies. Male and female genitalia are described with associated structures (dorsal genital papillae - male; epimeral plates - female) and assessed in terms of variation of spacial arrangement. All of these structures plus the characteristics of the egg shell are compared with older accounts (esp. Hirst, 1919) and discussed with respect to their validity in species discrimination. (Supported in part by NIH Grant 5 TOI AI-226).

28

How Many Species Has Salmincola, a Genus of Copepods Parasitic on Fishes?
Z. KABATA

Detailed morphological study of the genus Salmincola resulted in re-definition of the genus and addition to it of three species of Achtheres. In spite of this addition, the number of 29 species recorded in the literature is reduced to 15, possibly 14.

Comparison of morphological details shows that the second antennae and maxillipeds are taxonomically the most important appendages. Gross morphological features (shape of trunk, etc.) should be used with caution and only as corroborating evidence.

The genus is subdivided into two subgenera, one parasitic mainly on Salmonidae, the other exclusively on Coregonidae. Salmincola has five circumpolar species and seven known from the Palearctic only. Two species occur in both the Palearctic and Nearctic (one on the American and Asian shores of the Pacific, the other on the European and American shores of the Atlantic). Only one species is exclusive to North America.

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Monofactorial Inheritance of Susceptibility of Aedes aegypti to Plasmodium gallinaceum, W. L. KILAMA* and G. B. CRAIG, JR.

Various strains of Aedes aegypti infected with Plasmodium gallinaceum showed great variability in number of oocysts per midgut. From two strains with individuals giving few oocysts, two refractory lines have been established. Oocyst counts for susceptible lines are generally above 50, whereas refractory lines usually give no oocysts (maximum of 10).

Reciprocal crosses between refractory and susceptible strains give susceptible progeny in the F_1. Backcrosses to the refractory parent gave a 1:1 segregation ratio of susceptible to refractory phenotypes. Backcrosses to the susceptible parent gave nothing but susceptible progeny.

From crosses between a refractory strain and a susceptible strain with genetic markers, it has been demonstrated that the locus for plasmodium-susceptibility (pls) is on linkage group II, between the loci for Silver-mesonotum (Si) and Dieldrin-resistance (Dl).

The approximate linkage map is:

Si — 8 — pls — 17 — Dl

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Immersion Interferometry of Plasmodium berghei and Plasmodium vinckei, MICHAEL J. AUTUORI* and C. W. LACAILLADE

Soluble cytoplasmic protein concentration was measured in living erythrocyte host cells and cell-free parasites under various experimental conditions using the technique of immersion interferometry (Barer and Joseph, 1954).

The mean protein concentration (MPC) of populations of host cells studied during patent infection is distinctly different for the two species. The difference is believed to reflect the host cell preferences of the two parasites. The MPC of populations of cell-free P. berghei parasites is not significantly different from that of cell-free P. vinckei parasites. The downward trend in the MPC for P. berghei host cells during patent infection is similar to but greater in magnitude than that noted in the MPC of reticulocytes from repeatedly bled mice. MPC, proposed as a new parameter applicable to malarial study, unites with the classical parameters (percent reticulocytes and anemia) to indicate that the reticulocyte response in P. berghei infection is qualitatively different from the reticulocyte response to repeated bleeding in uninfected mice.

In vitro incubation of P. berghei and P. vinckei host cells at 38°C. in serum enriched with glucose demonstrated that both parasites destroy approximately one fifth of host cell hemoglobin during a twenty four hour period. Evidence is presented which suggests that the parasites affect the fluid content of their host cells. Host cells appear to take up fluid from the medium.

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Folate Metabolism in Plasmodium lophurae
EDWARD G. PLATZER

The activity of dihydrofolate reductase was investigated in duck erythrocytes infected with Plasmodium lophurae, uninfected erythrocytes, and isolated parasites. Dihydrofolate reductase activity in erythrocytes infected with small uninucleated trophozoites was similar to uninfected erythrocyte activity but was several fold greater in erythrocytes with large multinucleated parasites. Enzyme activity was present in isolated multinucleated parasites and was much higher than that found in normal erythrocytes on a per cell basis. The molecular weight of P. lophurae dihydrofolate reductase was much greater than that of the erythrocyte dihydrofolate reductase as shown by gel chromatography. Duck erythrocyte and liver dihydrofolate reductases were strongly inhibited by pyrimethamine, but P. lophurae dihydrofolate reductase was the most sensitive. These distinct differences in host and parasite dihydrofolate reductases demonstrated the independent nature of tetrahydrofolic acid synthesis from dihydrofolic acid in P. lophurae.

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Vital Considerations Concerning Purines, Pyrimidines and Amino Acid Incorporation Into Nucleic Acids and Proteins of Plasmodium berghei
C. LANTZ,* K. VAN DYKE and L.H. SAXE

Recent studies (Bungener; Van Dyke; Sherman) have indicated that purines and not pyrimidines are obtained exogenously by the malarial parasite. Pyrimidines can penetrate the red cell membrane yet are not utilized by the parasite. The parasite can synthesize pyrimidines; its entire pathway of pyrimidine biosynthetic enzymes appears to be intact (Sherman, 1969; Van Dyke, 1968). That the parasite must biosynthesize pyrimidines is shown by pyrimidine impenetrability through the parasite membrane. This same parasite membrane allows purines to be taken up and incorporated into nucleic acids (5-15% of added adenosine) at an extremely high efficiency. Attempts at measurement of amino acid incorporation into parasite protein failed because of the active amino acid incorporating ability of contaminating white cells. Removal of buffy coat (3x) still resulted in incorporation into white cell protein of 50% of the activity of a mixture of ^{14}C amino acids. Pyrimidines also incorporate into white cell nucleic acids and some recent studies have been vitiated because of this host cell contamination.

(Supported by U.S. Army Medical Contract #DA-49-193-MD-2767)

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Phagocytic Activity of Splenic Macrophages in Rodent Malaria, JOYCE S. CHOW and JULIUS P. KREIER*

Phagocytosis of erythrocytes and parasites by splenic macrophages is prominent in animals with malaria. We are studying the nature of the changes in the splenic macrophages in malaria in an in vitro system. The system is designed to yield information on both specificity and magnitude of activation of the macrophages. Spleens are removed from non-infected or acutely infected rats, teased apart, and the freed cellular pulp put into a glass bead column. The column is flushed to remove cells other than macrophages from the beads, and chromium-51 labeled erythrocytes are introduced. The relative retention of parasitized or non-parasitized erythrocytes by macrophages from normal or acutely infected rats is then determined.
The work is currently in progress. The initial 11 runs using macrophages from spleens of infected rats indicate that there is at least threefold greater retention of erythrocytes from infected rats than of erythrocytes of non-infected rats. This result indicates some specific change either in the macrophages or in the erythrocytes of the diseased rats.

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Maltose Metabolism of Trichomonas gallinae (Rivolta, 1878), James J. Daly

The utilization of maltose and glucose by T. gallinae (Jones' Barn strain) was compared by in vitro metabolic studies. Greater total growth was obtained at 24 hours (37°) with maltose rather than glucose in two media, STS (Simplified Trypticase Serum) and CPLM (Cysteine, Peptone, Liver, Maltose or Glucose). In STS medium the generation times with maltose were shorter than those with glucose. In CPL medium the generation times with maltose were approximately equal to those with glucose but the exponential phase was longer with the maltose supplemented medium. Stimulation of the production of CO2 and H2 gases above the endogenous levels was not significantly different between the two sugars. Substrate uptake, as determined by the disappearence of reducing sugar, showed equimolar utilization. Analysis of the glycogen content of cells revealed that maltose has a greater glycogen sparing effect than glucose. Results remained relative in the presence or absence of nutritional nitrogen (Trypticase). All experiments (except growth) were done with the Warburg respirometer, at 37°, in a gas phase of 100% N2, and in a milieu of Krebs-Ringer phosphate solution (pH 7.0). The results indicate that the greater total growth obtained in the presence of maltose rather than glucose may be related to the increased glycogen content and that this confers some energy saving advantage in growth. (Supported by a pre-doctoral fellowship, N.I.H. grant no. 5FL-GM-35, 087-02)

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Effects of Catecholamines on the Parasitemia

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Levels of Trypanosoma rotatorium in Rana clamitans, G. MASON*, and J. R. SEED.

Preliminary studies have revealed that the parasitemia levels of T. rotatorium in the peripheral blood of green frogs from Louisiana display a 24-hour periodicity. (Southworth, et al, 1968, J. Parasitol., 54: 255). Investigations into the effects of host physiology on the parasitemia have indicated that physiological stress applied to the host may alter the amplitude of this cycle. Physiological doses of epinephrine and norepinephrine were injected at 0800, 1200, 1600, and 2000 hours. The responses of the parasitemia levels to these agents varied with the time of injection. If the changes in parasitemia levels normally observed are due to catecholamine levels alternating with the time of day, inhibition of catecholamine biosynthesis would be expected to alter the cycle so that the parasitemia levels would remain constant. Inhibitors of catecholamine biosynthesis were injected at 0900, 1200, and 1500 hours. Though the amplitude of parasitemia was altered somewhat by the inhibitors, the results indicate that the catecholamine titers in the host are not the primary stimulus for the cycle. Further studies are in progress to determine the site of action of the catecholamines by the use of α- and β-adrenergic blocking agents. (This study was supported by N.S.F. grant GB-7401).

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Biochemical Changes in Livers of Guinea Pigs Infected with Trypanosoma gambiense, Y. MARCIACQ* and J.R. SEED

Previous work has shown that guinea pigs infected with T. gambiense die with hypoglycemia and liver glycogen depletion. To determine whether this condition is due to a true metabolic disturbance of the liver, the activities of the four liver enzymes involved in the direct utilization of glucose-6-phosphate were studied. Results have shown that activities of phosphohexoseisomerase and glucose-6-phosphate dehydrogenase remained normal whereas phosphoglucomutase activity was slightly decreased and glucose-6-phosphatase activity greatly decreased. An endogenous inhibitor of glucose-6-phosphatase has been found in homogenates of infected livers. It is possible that this inhibitor could account for the lowered glucose-6-phosphatase activity found in terminally infected livers. This inhibitor has been partially purified and characterized. Studies using bacterial pyrogen-free trypanosome extracts injected intracardially into guinea pigs have shown that, after two hours, an endogenous inhibitor is present and liver glucose-6-phosphatase activity is definitely lowered. The observed pathology is suggestive of a true liver disturbance of guinea pigs infected with T. gambiense. (Supported by DA Contract No. 49-193-2817 from the U.S. Army Medical Research and Development Command).

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Thymidine Kinase in Culture and Bloodstream Forms of Trypanosoma (Trypanozoon) rhodesiense.
P. L. CHELLO* and J. J. JAFFE

Thymidine kinase activity was detected in extracts of bloodstream and culture forms of T.(T.)rhodesiense. The trypanosomal thymidine kinases were partially purified by ammonium sulfate fractionation and comparative studies indicated that the enzymes from both sources had closely similar properties. Both were heat-labile and both were maximally active at pH 8.0. Michaelis constants of 2.7×10^{-5}M and 6.6×10^{-5}M for thymidine were obtained with the culture and bloodstream form kinases, respectively. In order of decreasing affinity, 5-iododeoxyuridine, trifluorothymidine, and 5-fluorodeoxyuridine were competitive substrates, but uridine and deoxyuridine were ineffective. The requirement for a phosphate donor was best met by adenosine triphosphate, followed by the triphosphates of uridine, guanosine, and cytidine. Both enzymes required magnesium for optimal activity. Basal trypanosomal thymidine kinase activity was augmented in the presence of adenine or guanine mononucleotides and was decreased in the presence of cytidine mononucleotides. Deoxycytidine diphosphate, found by others to activate E. coli thymidine kinase and to have no effect upon mammalian thymidine kinase, inhibited the trypanosomal kinases. The two trypanosomal thymidine kinases were equally susceptible to feedback inhibition by thymidine triphosphate. However, they were apparently much less susceptible to this agent than the thymidine kinases from bacterial and mammalian sources. (Supported by NIH Grant CA-08114-05.)

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Isolation and Properties of Phosphohexose Isomerase from Trypanosoma cruzi,
E. L. RISBY

Phosphohexose isomerase activity has been found in both soluble and particulate fractions of T. cruzi extracts. This enzyme has been purified some 50-fold from soluble extracts by a combination of ammonium sulfate fractionation and DEAE Sephadex column chromatography. Partially purified phosphohexose isomerase has the following properties: A) no metal ion requirement; B) inhibited by Ca^{++}, Co^{++}, Mn^{++} and PHMB; not inhibited by Mg^{++} or pyrophosphate; C) pH optimum 8.0-8.6; D) T^{o} optimum 50°C. T. cruzi phosphohexose isomerase differs from the analogous enzymes of blood stream trypanosomes with respect to pH, T^{o}, and metal ion effects. These enzymes show serological identity. (Supported in part by NIH Grant 5-S01-FR05422-08)

ABSTRACTS

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Cytochemical Identification of b-galactosidase, Aldolase, Phosphorylase, and Lipids in Eimeria stiedae. JOHN C. FRANDSEN

Using 5-bromo-4-chloro-3-indolyl-b-D-galactoside as substrate, b-galactosidase was identified in endogenous stages. The largest amounts of reaction product developed in female gametocytes and unsporulated oocysts, being closely associated with the plastic granules in the female cells. Activity of fructose phosphate aldolase was detected in schizonts, merozoites, male gametocytes, and unsporulated oocysts, but it was absent from most female gametocytes and when present occurred in very small amounts. Activity of a-glucan phosphorylase was identified in schizonts, merozoites, gametocytes, and unsporulated oocysts. Conclusions regarding the types (e.g., la and lb) of phosphorylase and mechanisms of interconversion of the types could not be drawn. Staining of endogenous stages with Sudan black B and 3,4-benzpyrene and of sporozoites with these stains and oil red O has revealed heavy accumulations of lipid in female gametocytes and unsporulated cocysts. The apparent pattern of lipid distribution in sporozoites varied according to the stain used, little lipid being visualized with oil red O, more with Sudan and 3,4-benzpyrene.

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Economic Significance of Coccidiosis in Calves
PAUL R. FITZGERALD* and M. E. MANSFIELD

Losses in poultry due to coccidiosis are well known but losses in cattle caused by coccidia have not been recently nor adequately determined. In the present study inoculation of Holstein-Friesian calves with sporulated oocysts of Eimeria bovis caused mild to severe infections. Beginning at patency food and water consumption of severely affected calves dropped much below that of the controls. This trend continued for about 6 weeks with the water and for about 2 1/2 months with the food. There was a slow recovery until at the end of these periods the previously affected calves were consuming about the same amounts of food and water as the controls. Beginning a few days after the start of patency the weights of severely affected calves began to decline. After patency weight was gained at a rate slightly less than that of the control calves. This trend continued for several months even though the food and water intake was comparable to that of the controls. The difference in weight between severely affected calves and the uninfected controls was about 60 pounds.
There was a reduction in serum protein beginning shortly after the patent period started. The reduced protein levels persisted for about 6 weeks then returned to the preinfection levels. The time required and the amount of additional food needed by convalescent animals will be calculated to ascertain how they compare with control calves.

41

Toxoplasma gondii: DNA Biosynthesis and Characterization.
A. GELDERMAN, J. PERROTTO* and M. LUNDE
National Institutes of Health
Bethesda, Maryland

Toxoplasma DNA was isolated and characterized as to genome size and percent guanine-cytosine content. Purine and pyrimidine biosynthesis of DNA was investigated by measuring the incorporation of free bases and base precursors. The results indicate that the parasite relies on the incorporation of free adenine for purine synthesis while pyrimidines are synthesized de novo. These biosynthetic pathways of DNA synthesis may explain why the parasite requires an intracellular environment.

42

Progress Report on the International Clearing House Program for Parasitological Material, FREDERICK H. MILLER and IRVING ABRAHAMS*

This program, now in its third year, was established to make available Parasitology teaching materials. The "International Clearing House" serves as a focal point for the voluntary exchange of specimens. When such materials are received they are catalogued, and many are processed to slides. Upon request these are supplied for teaching purposes. A total of over six thousand slides and specimens have been distributed from the clearing house. A summary of the clearing house activities to date will be presented and the importance of the continued cooperation of parasitologists will be emphasized. Lists of current materials ready for distribution will be available.

43

Wildlife Reservoirs of Capillaria hepatica (Bancroft, 1893). GENE B. SOLOMON* and CHARLES O. HANDLEY, JR.

The livers of 997 rodents and insectivores trapped in four major habitats (rocks, forest, field, stream bank) at 24 study areas in the vicinity of the Mountain Lake Biological Station (Virginia) were examined macroscopically and by the tissue press technique for the presence of Capillaria hepatica. Eighty-nine mammals were found to be infected. Seven host species, four of which appear to be new host records, were represented. Infection was concentrated in varying degree in rocky areas, and the rate of infection diminished rapidly in adjoining forests and fields. Our findings suggest that the parasite is maintained in nature in pockets of infection involving interaction of host and habitat. Other hosts and habitats of secondary importance may provide communication between the major foci of infection.
(Supported in part by NSF (GB-3439) summer post-doctoral fellowship awards and by a Grant-in-Aid of Research from the Society of Sigma Xi.)

44

Distribution of Meningeal Worm (Pnumostrongylus tenuis) in the Southeastern United States.

Annie K. Prestwood* and James F. Smith.

From 1960 through early 1969, 2409 white-tailed deer (Odocoileus virginianus) were examined for Pneumostrongylus tenuis. Animals originated in 137 counties of 13 southeastern states and St. Croix of the U. S. Virgin Islands. Meningeal worm was present in 1197 (49.7%) deer collected in Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Tennessee, Virginia, and West Virginia. Infected deer were not found in South Carolina and St. Croix. In the Southeast, meningeal worm appears to occur mainly in deer of oak-pine subclimax and climax deciduous forests. It was not found in areas having a sandy soil and a predominately pine forest. (This study was supported by an appropriation from the Congress of the Congress of the United States with funds administered and research coordinated under the Federal Aid in Wildlife Restoration Act (50 Stat. 917) and through Contracts Nos. 14-16-0008-676 and 14-16-0008-777, Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior.)

45

Influence of Weather on Intensity of Infection of Bighorn Sheep with Lungworms of the Genus Protostrongylus, DONALD J. FORRESTER

During a ten-year period (1959-1968) lungs from 157 Rocky Mountain bighorn sheep (Ovis c. canadensis) were examined for infections by the lung nematodes, Protostrongylus stilesi and P. rushi. All lungs were obtained from rams over three years of age which had been shot during September, October, November, or December of each year by special-permit hunters in the Sun River game area of western Montana. Year to year changes in intensity of infection were determined by measuring the surface areas of lesions caused by P. stilesi and by counting the numbers of adult P. rushi for each lung. The variations were studied in relation to weather conditions during the previous months. The intensity of infection each Fall was directly proportional to the amount of precipitation during the preceding April, May, and June. (Supported, in part, by National Science Foundation Grant G-19422 and the Montana Fish and Game Department.)

46

Skrjabinema ovis from Angora Goats in Texas, MARVIN LADERMAN* and V. J. THEODORIDES

Skrjabinema ovis (Skrjabin, 1915), Werestchagin, 1926, was found in the large intestines of 8 Angora goats from Junction, Texas. A total of 60 animals were examined. The female S. ovis measured 5.45 mm in length and 0.076 mm in width at the head. The males measured 2.51 mm in length and 0.041 mm in width at the head. This species has been reported for the domestic sheep and goat and the bighorn sheep (Schad, G. A., 1959, Proc. Helminthol. Soc. Wash., 15:138-147) and for the mountain sheep in New Mexico (Allen, R. W., 1955, J. Parasit., 41:583-587). We believe that this is the first report of S. ovis from Angora goats in Texas.

47

Food Attraction of Coprophagous Beetles Serving as Intermediate Hosts for Spirurids of Swine, G. T. FINCHER* and T. B. STEWART

Many adult coprophagous beetles of the family Scarabaeidae serve as intermediate hosts for Spiruridae of swine: thick stomach worms, Physocephalus sexalatus and Ascarops strongylina; and the gullet worm Gongylonema pulchrum. These parasites are of economic importance to swine producers and they can infect wildlife. Since other susceptible animals are commonly present near swine habitats, a study of food preference of dung beetles was made. Beetles were attracted to pit traps by baiting each trap with the feces of one of 12 different animals including man. Replicas were made in 3 habitats. Most dung beetles were captured in traps baited with swine feces. Swine and opossum feces attracted 57.7% of all beetles captured and 74.7% of the Phanaeus species which are the major intermediate hosts of swine spirurids. Overall the data demonstrate that dung beetles and in particular Phanaeus vindex and P. igneus are attracted more strongly to feces of swine than to feces of the other animals tested. Some form of evolutionary adaptation may be inferred from the fact that opossum feces had an attraction index greater than that of any other animal feces except swine. As domesticated animals are relatively new when compared to native wild animals in this country, perhaps some dung beetles are now in the process of adapting to feces of introduced animals and becoming less dependant upon wild animal feces.

48

Effect of Stocking Rates on Parasitism of Steers on Continuously vs. Rotationally Grazed Winter Temporary Pasture. H. CIORDIA

Tests were carried out during 3 consecutive grazing seasons, using winter pastures without supplemental feed. Each year, one group of steers (Group I) continuously grazed a pasture, and another group (Group II) rotationally grazed a comparable pasture on a 4-way system. Both groups

maintained the same average stock rate (1.1/acre) throughout the grazing season. A third group (Group III) also grazed rotationally, but with a varying stocking rate, dependent on the condition of the pasture (average stocking rate for the 3 years was 1.35/acre). Although not statistically significant, a higher average number of nematodes were recovered from steers from Group I than from Group II. The steers from Group III had the highest average number of parasites, indicating that an increase in the stocking rate of the pastures tended to be more conducive to parasitism than the type of grazing system used. Although the steers from the pasture having the greatest stocking rate made the lowest average daily weight gains, the pasture produced a higher total weight gain per acre and resulted in a greater net return. The mean parasite populations of each group of steers varied significantly with year, being directly related with the length of the grazing season.

49

Development, Migration and Survival on Pasture of Gastrointestinal Nematodes of Cattle -- Summer Contamination, AARON GOLDBERG*

Fecal pats containing eggs of Ostertagia ostertagi, Cooperia punctata or Oesophagostomum radiatum were deposited on pasture at Beltsville, Maryland, at the beginning of summer (June 24, 1968). Initial, peak and final recovery of infective larvae from the pats occurred 0.5 to 1, 1 to 2, and 8 to 9.5 weeks later, respectively. Initial, peak and final recovery of infective larvae from the herbage occurred at 2, 2 to 4, and 8 to 24 weeks, respectively. Eighty-one to 91% of the larvae recovered, per unit weight of herbage, was within 5 inches of the pats. The percentage development for O. ostertagi was considerably higher than that of the other species, but its migration onto the herbage was not as great as in previous trials with this species begun in May and October.

50

Effects of Repeated Desiccation and Hydration on Infective Haemonchus contortus Larvae.
K. S. TODD*, N. D. LEVINE and C. C. WHITESIDE

Third stage Haemonchus contortus larvae were obtained by incubating feces from monospecifically infected sheep. The larvae were collected by the modified Baermann technic, washed and placed in tap, distilled or triple distilled water. Approximately 200 larvae were put in each of a series of petri dishes and desiccated for 1-10 days at relative humidities of 30, 50, or 70% at the following temperatures: 20, 30, 40 and 50 C. The samples were removed daily, rehydrated in the same type of water in which they had originally been placed, and after 4 hours the motile larvae were counted. The larvae were then again desiccated. This procedure was repeated daily until all of the larvae were dead. Best survival was at 20 C and 75% relative humidity; some larvae survived over 80 days when desiccated repeatedly under these conditions. At all temperatures and relative humidities, control and desiccated larvae survived better in distilled or triple distilled water than in tap water. Preliminary work indicates that 3rd stage Trichostrongylus colubriformis larvae are not as resistant to repeated desiccation as are H. contortus larvae. (Supported in Part by Training Grant AI-00033 and Research Grant AI-06197 from the National Institutes of Health.)

51

Evaluation of the Baermann Technic for Infective Larvae of Haemonchus contortus, N. D. LEVINE*, K. S. TODD, JR. AND C. C. WHITESIDE

The effects of time, temperature, liquid vehicle, light, size of funnels, weight and length of grass, and type and amount of soil used for baermannization were determined for 3rd stage larvae of Haemonchus contortus. More larvae were recovered at temperatures between 4 and 25 C than at 30-50 C. Fewer larvae were recovered from detergent solutions than from water or physiologic salt solution; the latter two yielded essentially the same number of larvae. There was no significant difference in the number of larvae recovered in the light or dark. More larvae were recovered from short lengths and small amounts of grass than from longer and greater amounts of grass. Greater numbers of larvae were obtained from loose sandy soils than from compact clay soils. The most significant factor in determining the percentage of larvae recovered was the size of the funnel used for baermannization. A nearly perfect negative linear correlation was obtained when comparing percent recovery with funnels of 7.5, 10, 12.5, 15, 25 and 30 cm diameters. (Supported in part by Training Grant AI-00033 and Research Grant AI-06197 from the National Institutes of Health).

52

Anatrichosoma spp. in Old World Non-human Primates.
HELEN DAVIS CONRAD* and MING M. WONG

Anatrichosoma cutaneum (Trichosoma cutaneum) has been reported from skin lesions of Macaca mulatta and of two human cases as well as from the nasal mucosa of M. mulatta. A. cynomologi has been found in the nasal mucosa of a few M. irus. After finding, at necropsy, a much higher incidence of infection than was indicated by the infrequent presence of ova of these Trichosomoididae in the feces, a simple, nasal swab technique was designed and utilized in routine parasitological surveys at the NCPB laboratory since the summer of 1967. Of the 11 species of Old World non-human primates examined by the nasal swab method M. irus, M. mulatta, M. nemestrina and Cercocebus atys were found to be natural hosts for Anatrichosoma spp. The latter two species are new host records. Comparison of fecal concentration, nasal swab and

necropsy nasal mucosa dissection gave an average of 2% by fecal, 17% by nasal swab and 43% by necropsy examinations of Anatrichosoma infection in these 4 species of monkeys. The infection incidence was highest for M. nemestrina by each of the three methods. Unisex infections varied from 1 to 20 worms and when both sexes were present the ratio between them varied. Detailed morphological studies being done indicate there are more than one species of Anatrichosoma. Embryonated ova, incubated for different lengths of time have been fed to laboratory born juveniles. The life cycle is thought to be a direct one.

53

Anatrichosoma Infection in African Monkeys.

THOMAS C. ORIHEL

A survey of more than 150 monkeys at the Delta Primate Center revealed that nematodes belonging to the genus Anatrichosoma, previously reported only from Asian macaques i.e., rhesus and cynomolgus monkeys, also parasitize African primates. The parasite was found in the red patas monkey (Erythrocebus patas), talapoin (Cercopithecus talapoin), green monkey (Cercopithecus aethiops), mangabey (Cercocebus galeritus) and the baboon (Papio sp.) all from the African continent. The adult parasites in each of the primate host species were located in the nasal mucosa usually in the malpighian layer or occasionally in the dermis. On rare occasion, the adult worms were found in what was regarded as an ectopic location. Strings of eggs were found on the surface of the nasal mucosa but were rarely seen in the feces even when a variety of techniques for fecal examination were employed. Swabbing of the nasal mucosa proved to be the most effective method for diagnosis of infections.
(Supported in part by NIH Grants AI 06828 and FR 00164, DRFR, ARB.)

54

Further Studies on Filariasis in Dogs as Determined through Blood Studies,

R. N. GARCIA, J. W. WARD* and R. P. DODDS

Fresh blood, hemolized blood and stained blood smears were studied from 2,792 dogs in order to determined the presence of microfilariae of Dirofilaria immitis and Dipetalonema reconditum. The experimental animals were obtained from an area extending from Memphis, Tenn., to Jackson, Miss. and to New Orleans, La.
Examination of blood of 1,521 dogs up to 10 years of age showed 463 (30.4%) positive for microfilariae without specific differentation. Examination of 1,271 dogs up to 5 years of age showed 231 dogs (18.1%) positive for microfilariae of D. immitis and 92 dogs (7.2%) positive for microfilariae of D. reconditum. Studies on seasonal variation of microfilariae show that of 184 dogs examined in March 48 or 26% were infected with microfilariae of D. immitis and 18 or 9.8% were infected with microfilariae of D. reconditum.

55

Survey of Helminth Parasites of Dogs from Brazos County, Texas,

J. O. COSTA*
T. J. GALVIN and R. R. BELL

Helminth parasites were recovered from 44 dogs of mixed breeding collected from the Bryan - College Station area. Eight species of helminths were identified. These species and the prevalence of infection are as follows:

Ancylostoma caninum	-	90.9%
Dipylidium caninum	-	47.7%
Toxocara canis	-	27.2%
Trichuris vulpis	-	25.0%
Taenia spp.	-	18.2%
Dirofilaria immitis	-	4.5%
Spirocerca lupi	-	2.3%
Uncinaria stenocephala	-	2.3%

Twenty-one dogs were examined for Dipetalonema spp. (8 by Knott's technique for microfilaria and 13 by Dunn's technique for adults) without positive results. The heart and lungs were not examined in 3 cases, and in a fourth case the heart and esophagus were not examined. The hookworm A. caninum, was the most common helminth with respect to the number of animals infected and average worm burden. Uncinaria stenocephala is reported from Texas for the first time.

56

Geographical Distribution and Seasonal Incidence in the Helminth Parasites of the Starling, Sturnus vulgaris L.,

JAY D. HAIR

Seventy-five species of helminths, representing 49 genera, have been reported from starlings by numerous authors in different parts of the world. The list comprises 26 species of Trematoda, 13 of Cestoda, 27 Nematoda, and nine Acanthocephala. In all instances, more species of helminths have been reported from starlings from the eastern than from the western hemisphere. The seasonal incidence of gastrointestinal parasitism was studied in over 400 starlings from western South Carolina over a 13 month period. Records of incidence and burdens of the various species of helminths recovered show that definite seasonal helminth population cycles exist. The helminth faunas were predominated by the cestode Hymenolepis farciminosa and an acanthocephalan Plagiorhynchus formosus which were most numerous during the spring and summer months. Of these two groups, cestodes were the most common, and were recovered from birds examined each month, whereas there were five months during the winter in which no acanthocephalans were found.

ABSTRACTS

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The Teaching of Parasitic Diseases: Then and Now. K. L. HUSSEY. ASP - ASTMH Symposium.

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Experimental Parasitology and Tropical Medicine at Brown. ALFRED W. SENFT. ASP - ASTMH Symposium.

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Influence of Morphology and Ecology of the Second-Year Medical Student on the Knowledge Transport System. DONALD V. MOORE. ASP - ASTMH Symposium.

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Parasitic Diseases Teaching Experiences in Medical School. JOHN H. CROSS. ASP - ASTMH Symposium.

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Parasitic Diseases a Part of Medicine. HAROLD W. BROWN. ASP - ASTMH Symposium.

62

Junctional Nodules in Schistosomiasis: Development of Granulomatous Nodules at the Mesenteric Attachment to the Bowel

B. H. KEAN* and DAVID T. DENNIS

The presence of black nodules at the junction of the mesenteric attachment to the bowel may be striking in patients with S. japonicum disease. Histologic sections disclose masses of schistosome eggs separated by some fibrous tissue. Since the pathogenesis of these nodules was not clear, an attempt was made to reproduce the lesions experimentally.

A group of guinea pigs was infected with S. mansoni cercariae, the animals sacrificed at 11 weeks, and the junction of the mesenteric attachment to the bowel was examined grossly and histologically. Granulomatous nodules were frequently present. A series of lesions starting with the presence of adults in the area alone and followed by the development of nodules at sites of egg deposition was demonstrated.

These observations in S. mansoni were confirmed in baboons experimentally infected with S. japonicum.

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Experimental Infections with Schistosoma haematobium in the Chimpanzee,

E. H. SADUN*, F. VON LICHTENBERG, A. W. CHEEVER D. G. ERICKSON and R. L. HICKMAN

Detailed parasitologic, serologic, clinical and pathologic studies were conducted in chimpanzees. Four animals were exposed to a single dose of 500 or 2,000 cercariae each and 4 others were exposed monthly to 100 or 250 cercariae each. All animals were necropsied 7 to 18 months following initial exposure. Viable eggs were found in the urine and feces of these animals without significant reduction in number for the duration of the experiment. Intravenous pyelograms, cystocopy and necropsy revealed dramatic pathologic changes, remarkably similar to those reported for heavily infected humans. There was a correlation between the eggs excreted in the urine, the number of worms in the pelvic circulation and the pathologic observations. Numerous inflammatory plaques found in the bladder mucosa were microscopically identifiable as composite granulomas, scattered throughout all layers. The ureters showed irregular fusiform swellings, displacement of their course and distinct hydroureter. The inflammatory plaques of the ureteral mucosa resembled those of the bladder and caused ureterectasis, hydroureter and hydronephrosis. The bilharzial plaque appeared to be characteristic of the S. haematobium pathology in the chimpanzee both in the urinary tract and in the rectosigmoid. The typical plaque was sharply circumscribed with raised borders, clearly outlined, velvety and raspberry-like. There was a correlation between the parasitologic findings, serum biochemical test results, clinical, radiologic and pathologic observations. Fluorescent and homocytotropic antibodies were detected, but the time development of the two antibodies was dissimilar.

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On the Evolution and Involution of Egg Lesions in the Liver of Horses Infected with Schistosoma japonicum,

H. F. HSU,* S. Y. HSU, J. R. DAVIS, and W. MERGNER

The horse is not only the first animal in which the existence of acquired immunity against S. japonicum has been demonstrated, but also the animal in which interesting schistosome egg lesions have been described. The egg lesions are characterized by the incomplete necrosis in their center and the thick fibrous capsule in their outer layer. The descriptions of these characteristics were usually based upon the egg lesions obtained in the chronic stage of infection. The biological processes of the evolution and involution of these egg lesions, however, have never been studied. The present paper is made for the study of these processes. Sections made from livers of 7 horses experimentally infected with S. japonicum (duration: 40-180 days) and from livers of 4 horses naturally infected in Japan (duration unknown) were studied. The results show that two kinds of biological processes of the egg lesions were going on in the same individual host. Under one kind of biological process, the egg lesions can be divided into 5 stages, as we previously described for the egg lesion in the

rhesus monkey infected with S. japonicum (non-reactive or weakly reactive stage, exudative stage, exudative-productive stage, productive stage, and involutional stage). Under the other kind of process, these 5 stages were modified by the participation of the action of eosinophils. However, the basic principles of the evolution and involution of the egg lesions were the same in both kinds of lesions.

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CYTOLOTY OF THE BILE DUCT IN MICE INFECTED WITH THE TAPEWORM HYMENOLEPIS MICROSTOMA

D.S. KARIN* and R. D. LUMSDEN

The bile duct hypertrophy characteristic of rodents infected with H. microstoma has been attributed to lymphocyte infiltration and hyperplasia of the glandular epithelium (Bogitsh, 1966, Trans. Am. Micr. Soc. 85: 372). In an attempt to further define the histopathology associated with this host-parasite relationship, sibling mice were each administered 6 cysticercoids per os and sacrificed at selected intervals over a period of 6 mo. Samples of the affected tissues were studied by electron microscopy and cytochemical methods. A typical inflammatory response manifest by leucocytic and lymphocytic infiltration of the peribiliary connective tissue is apparent within 1 week postinfection. Subsequent growth of the bile duct is largely a reflection of fibroblast proliferation and associated collagen synthesis. Certain of the lymphocytes differentiate into plasma cells which constitute characteristic elements of the stroma in long term infections. Concommitant with cessation of further bile duct enlargement, fibrogenesis terminates. At this time, the stroma consists of densely packed collagen, scattered fibrocytes, plasma cells, mast cells and leukocytes. In areas of physical contact between the parasite and lumenal surface of the bile duct, there is focal degeneration of host epithelial cells. The morphology of adjacent epithelial cells is normal, except for accumulation of large lipid deposits in older infections. Hypertrophy of alveolar glandular activity is suggested by extensive dilation of endoplasmic reticulum and Golgi cisternae. (Supported by grants from the NIH (AI 08673), NSF (GB 7276) and Career Development Award (to RDL) k04-AI 23449 from the USPHS).

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PATHOGENICITY OF GEOGRAPHIC STRAINS OF ANGIOSTRONGYLUS CANTONENSIS IN THE TAIWAN MONKEY.

J. H. CROSS* AND J. W. FRESH

SINCE LARVAE OF ANGIOSTRONGYLUS CANTONENSIS HAVE BEEN RECOVERED FROM HUMAN SPINAL FLUID IN TAIWAN AND NOT FROM PATIENTS WITH ANGIOSTRONGYLIASIS IN OTHER COUNTRIES, STUDIES WERE INITIATED TO DETERMINE WHETHER DIFFERENCES EXISTED BETWEEN GEOGRAPHIC STRAINS OF THIS PARASITE. A. CANTONENSIS WAS OBTAINED FROM TAIWAN, HAWAII, THAILAND, PHILIPPINES, INDONESIA, AND MALAYA AND MAINTAINED IN THE LABORATORY BY PASSAGES THROUGH THE AQUATIC SNAIL, BIOMPHALARIA GLABRATUS, AND LONG-EVANS RATS. INITIAL STUDIES WITH TAIWAN STRAIN SHOWED THAT INFECTIONS WITH AT LEAST 10,000 LARVAE WOULD KILL MONKEYS IN 7 TO 35 DAYS. MOST ANIMALS LOST WEIGHT, DEVELOPED A LEUCOCYTOSIS WITH EOSINOPHILIA AND EOSINOPHILIC PLEOCYTOSIS. AT AUTOPSY THE LUNGS WERE ATELECTATIC AND THERE WAS VASCULAR CONGESTION IN ALL ORGANS INCLUDING THE CENTRAL NERVOUS SYSTEM. IMMATURE WORMS WERE FOUND IN THE LEPTOMENINGES, BRAIN, AND CORD SUBSTANCE AS WELL AS IN THE CENTRAL CANAL OF THE CORD. WORMS WERE NOT FOUND IN ANY OTHER ORGAN. PATHOLOGICALLY, THE PARASITE APPEARED TO PRODUCE FOCAL HEMORRHAGE AND ABSCESSES IN EARLY INFECTIONS AND GRANULOMATOUS SPACE-OCCUPYING LESIONS IN OLDER INFECTIONS. EXCEPT FOR THE MALAYSIAN STRAIN, THE OTHER STRAINS OF A. CANTONENSIS TESTED HAVE BEEN SHOWN TO BE EQUALLY AS PATHOGENIC TO THE TAIWAN MONKEY AS THE TAIWAN STRAIN. ALTHOUGH MONKEYS GIVEN THE MALAYSIAN STRAIN DEVELOPED SYMPTOMS, AND IMMATURE WORMS WERE RECOVERED IN SPINAL FLUID, ALL ANIMALS SURVIVED THE INFECTIONS. IN ADDITION, SURVIVING MONKEYS WERE FOUND TO BE RESISTANT TO LETHAL CHALLENGING INFECTIONS WITH PATHOGENIC STRAINS. IT IS WORTHY TO NOTE THAT THE PATHOLOGICAL FINDINGS IN MONKEYS ARE SIMILAR TO THOSE FOUND IN A CHILD WHO DIED OF ANGIOSTRONGYLIASIS IN TAIWAN. (SUPPORTED IN PART BY BUREAU OF MEDICINE AND SURGERY, NAVY DEPARTMENT, FOR WORK UNIT MR005.09-0046B.)

67

Pulmonary Arterial Changes in Canine Dirofilariasis,

SI-KWANG LIU,* DALE A. YARNS, and ROBERT J. TASHJIAN

The main pulmonary arterial changes of canine heartworm disease (Dirofilaria immitis) were preocclusive dilatation and obstruction. The affected arteries were irregular, truncated, and saccularly dilated and had lost their gentle tapering and branching. In dogs with mild infections, the vascular lesions were most often unilateral or bilateral in the diaphragmatic lobar arteries and their branches. In dogs with moderate infections, dilatation and obstruction were always in the distal end of, and in one or two primary branches of, both diaphragmatic lobar arteries and in the right cardiac lobar arteries. The arterial lesions were occasionally seen in the right apical, left cardiac, left apical, and intermediate lobar arteries. Blood supply was diminished in the periphery of the diaphragmatic lobes. In dogs with severe dirofilariasis, marked saccular, preocclusive dilatation and obstruction were observed in all lobar arteries and their primary and secondary branches. Complete stoppage of contrast medium was seen in the periphery of the diaphragmatic lobes.

68

Lymphatic Involvement in Experimental Filariasis,

D. L. PRICE* and J. M. MORRIS

We have adapted the technique of lymphoradiography for studying the progress of pathologic changes associated with experimental filariasis that does not require the infected host to be killed and autopsied. Our procedure allows the identifi-

cation and isolation of the involved lymphatic vessels and accompanying nodes, their surgical removal, and the gross and histopathologic evaluation of tissue reaction. Applying this procedure to Brugia pahangi infected cats, we identified the specific lymphatics involved and resected these tissues for detailed study. In one experiment, a single popliteal lymph node was found to be enlarged approximately three times and its afferent vessels were dilated and tortuous. The efferent lymphatic was obstructed several centimeters from the affected node and in the lymphatic between this node and the area of obstruction, three adult B.pahangi were found. The next lymph node in the chain, a few millimeters distal to the obstructed lymphatic, was apparently unaffected.

Supported in part by Biodynamics Research Corporation Special Fund and in part by a Medical Research and Development Command U.S.Army research contract: Project No. 3A014501B71Q.

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Feeding Behavior of Adult Ancylostoma caninum and Related Blood Loss in the Host, ULRICH P. KALKOFEN

Feeding behavior of A. caninum was studied to define its relationship to associated blood loss. Wells' method of observing worms in vivo was used, and the observations extended to worms and tissue fixed in situ after various short periods. In addition, adult worms of one sex were either pipetted into the jejunum of dogs and recovered from 1 to 24 hours later, or were fed to dogs in gelatin capsules and recovered at one-day intervals. At necropsy all attached worms were excised together with adjacent intestine and fixed in situ. The histology of ingested tissue and of tissue surrounding the worms was used in reconstructing the probable sequence of feeding and related events. Feeding is essentially restricted to the mucosa. A nubbin of mucosa is sucked into the buccal capsule. The epithelium detaches and is carried into the worm's intestine along with blood flowing from ruptured vessels within the ingested bolus. The indrawn tissue becomes constricted at the buccal orifice as the mass of lamina propria is drawn from the surrounding areas toward the opening, radiating from there in fan-like fashion. The host tissue in the capsule of the worm is broken down and sucked into the midgut before a new "bite" is taken. Prolonged feeding in one location results in extensive trauma. Hemorrhage in the vicinity of the site of attachment becomes increasingly significant after the initial "bite", and appears to be of considerable importance in the manifestations of hookworm disease. (Supported by NIH grants AI-00002 and AI-04919).

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The Effect of Trichospirura leptostoma on the Pancreas of Callithrix jacchus, W. N. SMITH* and B. M. LEVY

Trichospirura leptostoma has been described from the pancreatic ducts of the white-eared marmoset, Callithrix jacchus, from Brazil. Varying degrees of chronic fibrosing pancreatitis was observed in association with the presence of the worms. Responses vary from slight fibrosis around the ducts to a chronic fibrosis throughout the entire gland. In the early stages, the fibrous connective tissue immediately around the ductal epithelium is infiltrated by scattered lymphocytes with occasional plasma cells and polymorphonuclear leukocytes. In the more extreme cases, the glandular epithelium is destroyed and replaced by well collagenized connective tissue in which there are foci of inflammatory cells, small islands of intercalary ducts, pancreatic alveoli, and islands of Langerhan's. It is questionable whether the remaining secretory cells of the pancreas function. The pancreatic islets are usually not involved in the fibrosing pancreatitis except in the most extreme cases. There was no correlation between blood sugar levels and the degree of fibrosis observed in the pancreas.

(Supported in part by grants DE-02232 and 5 F03 DE-35154-03 from the National Institute of Dental Research.)

71

Susceptibility of Several Insect Pests to Neoaplectana glaseri, C.P. TURCO*, S.H. HOPKINS, and W.H. THAMES JR.

Tests were conducted to determine if N. glaseri can effectively parasitize and kill certain insect pests. Nematodes were reared after the technique of Stoll in large, cotton plugged tubes with agar and peptone-beef heart infusion and supplemented with fresh sterile rabbit kidney. Larval forms of the insects tested were reared on a slight modification of a non-septic diet described by Shorey and Hale. Tests for parasitism were conducted in the laboratory and in a greenhouse to simulate field conditions ideal for the parasite. Tests showed the following results: (1) N. glaseri was able to enter the sugarcane borer, rice water weevil, spotted cucumber beetle, and some species of the white grub to complete its entire life cycle; (2) the fall armyworm was found to be resistant. The results show the nematode to be especially effective with insect forms that spend a great deal of their life cycle in direct contact with the soil.

(Supported in part by a Lamar State College of Technology Research Grant and a NASA Research Fellowship.)

72

An Abnormality of Nematode Cuticle.
G.J. JACKSON*, M.A. RUDZINSKA and D.D. DESPOMMIER,
The Rockefeller University, New York 10021

An abnormality, cuticular bubbling, was noticed in one of several lines of nematodes, Neoaplectana glaseri, after 23 years of cultivation apart from the source host, Popillia japonica grubs. The abnormality has been seen in all developmental stages, is particularly apparent in old or undernourished cultures and does not significantly interfere with the reproduction potential of well nourished cultures. Affected and normal worms have been examined by light and electron microscopy in order to describe the abnormality in detail. Temperature and drug cures have been attempted, so far with negative results.

73

Dog Infection with Ancylostoma caninum Comparison of Prepatent Period Following Infection of Puppies by Oral and Percutaneous Routes, WILLIAM M. STONE

The prepatent period following infection of puppies with Ancylostoma caninum during the first few weeks of life has been presumed to be indicative of the mode of infection, whether oral, percutaneous or prenatal. These prepatent periods were arrived at for the most part, by observation of animals designated as less than four months of age. This study compares the prepatent periods following infection of puppies of less than 10 weeks of age by oral and percutaneous routes. The prepatent period plus or minus two sample standard deviations as computed for oral infection was 13.4 ± 1 day and for percutaneous infection was 12.5 ± 2.4 days. This constitutes the lowest prepatent periods observed for oral and percutaneous infections. It supports the conclusion that no inferences as to route of transmission of infection can be made from such observation.

74

Migration Patterns and Development of Metastrongyloids in Carnivores, P.H.G. STOCKDALE* and ROY C. ANDERSON.

The routes of migration and development of Aelurostrongylus abstrusus in cats, Crenosoma vulpis in dogs, and Perostrongylus pridhami and Filaroides martis in mink, are described. A. abstrusus and P. pridhami migrate through the diaphragm and penetrate the pleural surface of the lungs within 24 hours after infection. C. vulpis reaches the lungs within 24 hours via the hepatic portal system, liver, hepatic vein, heart, and pulmonary circulation. F. martis requires two weeks to reach the lungs after migrating along the systemic arterial system (gastric, coeliac arteries, dorsal aorta) to the base of the heart, and then along the pulmonary arterial system; worms are subadult by the time they reach the lungs. The final moult of the four species is considered to be incomplete giving rise to the teguminal sheath.

75

Arrested Development of Haemonchus contortus in Lambs.

N. M. BLITZ* and H. C. GIBBS

Studies were undertaken to determine the importance of factors, other than acquired resistance, in the arrested development of H. contortus in sheep. In parasite-free lambs grazed for three-week periods on contaminated pasture during the summer and autumn, the proportion of parasitic larvae arrested at the early fourth stage increased from 1% in June to 100% in September. In similar lambs held in pens and simultaneously given (7) multiple doses of larvae, the proportion of arrested fourth stage larvae rose from 5% in June to a maximum of 36% in September, before declining to 11% in October. Neither the total worm burden nor the environmental regime under which the eggs and larvae were cultured apparently affected the proportion of parasitic larvae arrested in the lambs. Approximately 12,000 arrested fourth stage larvae recovered from three old ewes killed in midwinter (January), were surgically transplanted into the abomasum of two bred yearling ewes, raised parasite-free from birth. Their faecal nematode egg counts remained below 400 eggs per gram of faeces (e.p.g.) until 12-14 weeks after the transplantation took place and 4-5 weeks after lambing, at which time (April-May) they rapidly rose to over 2,000 e.p.g., thus duplicating the typical spring rise, or post-parturient rise seen in lactating ewes at this time of year.
(Supported by National Research Council Canada Grant A-3240.)

76

Larval Development and Transmission of Parafilaroides decorus (Nematoda: Pseudaliidae) in the California Sea Lion (Zalophus californianus). MURRAY D. DAILEY

P. decorus is a common parasite in the lungs of Z. californianus. In natural infections, first-stage larvae are shed with the excrement into tidepools at the breeding grounds. Fecal material

containing larvae is eaten by opaleye fish (Girella nigricans). Unexposed G. nigricans were infected in the laboratory and the larval development followed. The first moult occurred from 12-15 days post-infection in the intestinal mucosa. Second-stage larvae were found just beneath the longitudinal muscle layer. After 20 days larvae were found in the serosa and mesenteric adipose tissue. The second moult occurred from 25-30 days post-infection in those regions. All cuticles were retained. At 35 days post-infection, fish were fed to a young uninfected California sea lion. First-stage larvae were recovered after 21 days. This is the first life history report on lungworms infecting marine mammals. (Supported by U.S. Navy contract.)

77

Relation of Sex Balance of Angiostrongylus cantonensis to Location of Worms and to Lesions in Lungs of Rats, ELSA L. WINSOR

To investigate the relation of the sex ratio of Angiostrongylus cantonensis to the location of the rat host, ten or less juvenile adult worms of known sex were transferred from the brains of donor rats to the brains of uninfected rats. Shortly after the time of expected patency, the recipient rats were sacrificed to determine the location of the worms. Worms in bisexual infections with a balanced sex ratio (1-1, 3-3, 5-5) were never found in the heart but were usually in the left inferior lobe of the lung. In unbalanced bisexual infections (1-5, 2-5, 1-9, and 5-1, 5-2, 9-1) and in unisexual infections (1-0, 5-0, 10-0, and 0-1, 0-5, 0-10) worms were in the heart and were in lobes of the lung other than the left inferior lobe more frequently than in balanced infections. The predominate sex was found outside the left inferior lobe more often in unbalanced bisexual infections. Heart infection was more common when five or more worms were present. Obliterative endoarteritis to various degrees was present in all infections regardless of sex balance; parenchymal lesions varied in type and extent with the sex and number of worms present. The results suggest that the location of Angiostrongylus cantonensis and pathologic lesions in the host are determined in part by sex balance of the worms. (Supported by NIAID Grant AI-00002).

78

Infection of ticks with Dipetalonema viteae. GUILLERMO PACHECO*, MARK J. ATKINS and JOAN GURIAN

To determine the relationship between the number of microfilariae of Dipetalonema viteae in a sample of blood ingested by a tick when it feeds on an infected jird and the number of microfilariae in blood samples of known volume from the retro-orbital sinus of the same host, as well as the relationship between the number of microfilariae ingested and the number of infective larvae that develop in the tick, Ornithodoros tartakowskii were classified visually into small, intermediate and large, marked individually, weighed and fed on jirds with microfilaremia in one of three ranges. Individual ticks were reweighed immediately after the blood meal. Approximately 1/5 of the ticks in each group of 20 was sacrificed immediately after the second weighing and the number of microfilariae counted. The remaining ticks were stored in an insectary, dissected 28 to 38 days after the blood meal and the number of larvae recovered were counted.

Both the concentration of larvae in the tick immediately after feeding on an infected jird and the concentration of larvae in the tick 28 to 38 days after the blood meal can be described by functions of the concentration of microfilariae in the jird on which the tick fed. There was a one-to-one correspondence of the number of microfilariae in the sample of blood taken by the tick and the number observed in samples of blood from the retro-orbital sinus, without an upper limit. By contrast the data on concentration of larvae in the tick was asymptotic and approaches an upper limit as microfilaremia in the jird increases.

79

Observations on the development of Brugia pahangi in small laboratory animals. LAWRENCE R. ASH* and JOHN M. RILEY

Earlier workers have demonstrated that it is possible to obtain complete, although inconsistent, development of B. pahangi in small laboratory animals. This study reports on further attempts to determine the suitability of various rodents to serve as experimental models for this species of filarial worm. Patent infections were produced in two species of jirds, Meriones unguiculatus and M. libycus. Microfilariae were demonstrated in the peripheral blood of both species of jirds as early as 74 days. Adult worms, of normal size and appearance, were found in the epididymis and tissues of the pelvic region of M unguiculatus. (Supported by NIH Grant AI-07770 USPHS.)

80

Presidential Address. An Old Timer's Look at Parasite Physiology. THEODOR VON BRAND.

81

Introduction. PAUL P. WEINSTEIN. ASP Symposium

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Physiology and Biochemistry of Parasitic Protozoa. WILLIAM TRAGER. ASP Symposium.

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Comparative Energy Metabolisms of some Parasitic Helminths. HOWARD J. SAZ. ASP Symposium.

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Physiology and Biochemical Aspects of the Host-Parasite Relation. CLARK P. READ. ASP Symposium.

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Summation. ERNEST BUEDING. ASP Symposium.

86

Immunologic Studies on Schistosomiasis in Rhesus (Macaca mulata) Monkeys, SHIRLEY E. MADDISON, SADIE J. GEIGER, and IRVING G. KAGAN*

Infection of the rhesus monkey with Schistosoma mansoni results in protection against reinfection of this host. The mechanism of this immunity has not, as yet, been fully elucidated. In the present investigations, 23 rhesus monkeys have been studied over periods of up to 85 weeks. Some of the animals were exposed to 1,000 or 2,000 cercariae, and the course of infection was followed by weekly fecal egg counts. Various schedules were used with a number of animals receiving only antigenic extracts of S. mansoni adult worms. The humoral responses in all animals were assessed by 4 serologic tests. Reaginic antibody was detected by immediate-type skin reactivity and passive cutaneous anaphylaxis tests. Skin test sites were observed for possible delayed-type reactions. Protection was afforded by initial exposure to 1,000 cercariae in one group but not by pretreatment with antigen in the second group. Antibody response in both of these groups was somewhat variable. Exposure to 2,000 cercariae resulted in reactivity in all serologic tests and immediate-type skin reactivity. The "cercarien-hüllen reaktion", bentonite flocculation, and reaginic antibodies were more transient than were the complement fixing and hemagglutinating antibodies. The presence of humoral response did not correlate with the immunologic status of the host, and skin reactivity of the delayed type was not observed.

87

Nature of the Reaction of Antigen with Sensitized Lymphocytes in the Lymphocyte-Dependent Release of Histamine from Platelets of Rabbits Infected with Schistosoma mansoni, J. F. BARBARO* and M. J. SCHOENBECHLER

Schoenbechler and Barbaro recently demonstrated that lymphocytes from rabbits infected with Schistosoma mansoni reacted with antigen yielding histamine release from rabbit platelets (PNAS 60: 1247, 1968). Leukocytes from infected rabbits reacted with antigen washed free of excess antigen can induce histamine release from platelets. Leukocytes prepared in this way give maximal histamine release within 5-10 minutes; while those reacted with antigen in the presence of platelets require 15 minutes to achieve the same level. Ten minutes was required for the reaction of leukocytes with antigen at 37°C before removal of excess antigen to achieve maximal release of histamine. Leukocytes reacted with antigen for 15 minutes at 1°C and 22°C and washed before adding to platelets at 37°C gave little or no histamine release. Leukocytes reacted at 44°C with antigen, washed and added to platelets at 37°C gave distinctly less histamine release than those reacted with antigen at 37°C. The first step in histamine release is the reaction of antigen with the sensitized lymphocyte, and this step involves at least one temperature dependent reaction.

88

Immunization of Rhesus Monkeys against Schistosome Infection by Cercariae Exposed to High Doses of X-radiation, S. Y. Li Hsu*, H. F. Hsu, and J. W. Osborne

The most effective radiation exposures used for schistosome cercariae which subsequently immunize the animal have been reported to range from 2,500 to 3,000 R. Since inflammatory reactions caused by the schistosomula derived from irradiated cercariae exposed to these amounts of X-irradiation have been observed in the liver and lungs, it will be of interest to investigate the possibility of using a higher X-ray exposure (24,000 to 48,000 R) which will make the schistosomula perish in the less critical tissues such as the skin. The possibility of inducing acquired resistance by immunizing rhesus monkeys with Schistosome japonicum or S. mansoni cercariae exposed to such large amounts of X-irradiation will be investigated. Further interest in this study lies in the fact that some investigators have recently claimed that the migratory schistosomular stage contributed little to the development of host resistance. Since all immunizing cercariae exposed to such large amounts of X-irradiation will perish in the early schistosomular stage, the role of schistosomula in the induction of acquired immunity can be ascertained by immunizing monkeys with heavily irradiated cercariae. Four monkeys were given immunizing cercariae of S. japonicum; and two, of S. mansoni. The results show (1) that strong acquired resistance against schistosome infection in rhesus monkeys can be induced by previous immunizations with cercariae exposed to 24,000 or 48,000 R. and (2) that acquired resistance against schistosome infection can be induced exclusively from functional antigen produced following contact or deterioration of the schistosomular stage in the dermal tissue.

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Evidence of Indirect Antagonism by Schistosoma mansoni Against Development of the Echinostome,

89

Paryphostomum segregatum, within the Snail Host, H. K. LIM* and D. HEYNEMAN

Biomphalaria glabrata albino snails with well-established month-old infections of S. mansoni were exposed to P. segregatum miracidia. Development of the second parasite was delayed for about one week, as seen by appearance of the mother sporocysts and migration pattern of rediae. Sporocysts of P. segregatum in double infections were smaller and showed affected germ cells compared with controls. Penetration of miracidia appears unaffected; the effect is directed chiefly against developing embryos, primarily an inhibitory reaction.

P. segregatum miracidia were more rapidly immobilized in hemolymph of S. mansoni-infected snails than in control hemolymph at dilutions of 1:2, 1:4, and 1:8. Miracidia incubated in hemolymph of S. mansoni-infected snails, and then exposed to B. glabrata snails, showed lower infection rates than did control miracidia incubated in noninfected snail hemolymph.

These data support the view that inhibitory substances in infected B. glabrata have an antagonistic effect on a challenging trematode infection. Whether such substances originate from the parasite or from the host is not yet known.

(Supported by USPHS NIH Grant AI07054.)

90

On the course of infection by the schistosome trematode Trichobilharzia ocellata in its duck hosts as indicated by parasite egg passage, T.K.R. BOURNS*, M.E. RAU and J.C. ELLIS.

After being exposed to cercariae of Trichobilharzia ocellata by 90 min foot immersion, birds were kept on wire over 0.85% NaCl. Saline-washed fecal solids resuspended in water were placed in side-arm flasks from which miracidia were collected and counted. In black ducks which had been exposed once to massed cercariae, viable eggs were first passed on day 13, their numbers increasing until day 16 to 20, after which they decreased to 5% of peak value by day 38. Sporadic passage of low numbers of eggs occurred for at least one year. Patent infections were obtained in black duck, canvas-back, domestic Pekin and Khaki Campbell, American golden-eye, mallard, Mexican Tree duck, pintail, redhead, shoveller, green- and blue-winged teal, wood duck, and Canada goose, but gadwall, American widgeon, and coot were refractory. Birds infected when less than one week old showed infections whose time sequence was indistinguishable from that obtained when birds were infected at nine weeks. Exposure of black ducks to 10 to 25 cercariae for 51 consecutive days produced infections with erratic patterns of egg passage.

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Development of Trichobilharzia ocellata (schistosomatidae) in primary and challenge infections in Anas rubripes, J.C. ELLIS*, T.K.R. BOURNS and M.E. RAU.

Development of Trichobilharzia ocellata in the black duck, Anas rubripes, was observed in sections and tissue squashes after 90 min primary and challenge exposures to cercariae. As early as 19 hrs after primary exposure, schistosomulae reached the lung where they entered the bronchi, later to penetrate the epithelium and enter veins. Most had left the lung by day 4 although many returned to perish there on days 8 and 9. Worms appeared in liver at 24 hrs and accumulated there until day 8. Migration to the gut began on day 8 and egg-laying started on day 9. Adults were found deep in the T. mucosa until day 21 and eggs occurred in the gut wall until day 56. A small number of adults was found in liver 168 days after exposure. Functional immunity was apparently induced by the primary infection for, although schistosomulae of challenge infections penetrated skin and reached liver and lung, they failed to mature or to migrate to the gut.

92

Resistance of the black duck Anas rubripes to reinfection by the schistosome Trichobilharzia ocellata, M.E. RAU*, T.K.R. BOURNS and J.C. ELLIS.

Ten black ducks were exposed to massed cercariae of Trichobilharzia ocellata 16-23 days after the termination of egg passage resulting from primary infections. Eight of the birds exhibited complete immunity while the remaining two passed low numbers of eggs. All of 10 birds challenged 11 to 16 months after primary infection were totally resistant. Similarly, all of 9 ducks which had initially been exposed to 10-25 cercariae daily for 51 days exhibited solid immunity when challenged 70-100 days later. Eight-day-old adult worms injected into the vena magna of non-sensitized birds did not affect the course of infection produced when the recipients were exposed to cercariae the next day. When, however, similar birds were challenged by cercariae after 68 days, no second infection resulted.

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Host response of the flour beetle, Tribolium confusum, to single and challenge infection with Hymenolepis diminuta and H. microstoma, DONALD HEYNEMAN and MARIETTA VOGE*

Cysticercoids of H. microstoma developed as readily in flour beetles containing a prior infection with the same species, or after infection with H. diminuta, as did single-infection H. microstoma controls. Cross infection with H. microstoma following infection with H. diminuta also failed to show reduction in parasite numbers compared with controls. Some indication of reduction in the challenge infection was observed after H. diminuta reinfection and also with H. diminuta infection following H. microstoma infection.

Serial sections of beetles subjected to repeated infection and to cross infection showed no

structural alteration of parasites and no delay of developmental rate in the challenge infection. One to three layers of host cells were observed around some cysticercoids, but the host cell reaction was not consistent or uniform. Fine structure of the host-parasite interface showed that host cells do not come in direct contact with the cysticercoid surface. (Supported by NIH grants AI-07054 and AI-07332.)

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Immunogenicity of Ascaris suum Larval Metabolic and Somatic Antigens, JORGE GUERRERO* and PAUL H. SILVERMAN

These studies are part of a larger project designed to study the antigenic activity of metabolic and somatic antigens of third stage A. suum larvae in mice. Metabolic and somatic antigens were obtained from late third stage larvae removed from the lungs of infected mice and cultured for 5 days in supplemented Eagle's medium. Two intraperitoneal injections of antigens in 4% sodium alginate were given prior injection. Seven days after infection with 10,000 embryonated A. suum eggs injected per os the animals were killed, lung scores were recorded and the larvae were recovered from the lungs by Baermanization. The results showed that lyophilized metabolic antigens harvested from third stage A. suum larvae cultured in vitro caused a 74% reduction in the number of larvae recovered from the lungs when compared with control animals. (Supported in part by USPH Grant AI 05910).

95

Lymphocyte and Macrophage Population Kinetics in Ascariasis. E. J. L. SOULSBY*

Autoradiographs were prepared of impression smears and sections of tritiated thymidine-one-hour-pulse -labelled liver, lung, their draining lymph nodes and other lymphoid organs of guinea pigs infected with Ascaris suum. On initial infection phagocytosis by unlabelled macrophages was markedly increased in the liver, but a similar increase was not seen in immune animals. A marked increase in the population of labelled cells in the draining lymph node of the liver occurred 2 days after initial infection, reaching a peak at 7 days. By 4 days labelled lymphoid cells started to accumulate in the liver reaching a peak at 9 days. Such cells were probably derived from the local lymph node and from a dividing population in the liver. The response was local and more distant lymphoid tissue did not respond until some time later. The mediastinal lymph nodes responded 5 days after infection, showing an increased proportion of labelled cells. However, a progressive accumulation of unlabelled plasma cells occurred both in the mediastinal lymph nodes and the lung tissue. The response in the lungs further differed from the liver by showing accumulation of unlabelled macrophages and eosinophils. The difference in the response of liver and lungs is related to the basic mechanisms of immunity and immunopathology. (Supported by USPHS AI 06262).

96

Sequelae to Intravenous and Subsequent Oral Inoculation with Oesophagostomum radiatum in Cattle, HARRY HERLICH*

The fate of artificially exsheathed infective larvae injected into the jugular vein of cattle and the immunogenicity of this treatment were examined. Eighty thousand larvae were injected into 5 calves and within 2 weeks all developed dyspnea and inappetence. In 2 calves killed, 26 and 46 days after injection, the lungs were congested and covered with cheesy nodules. Live fourth-stage larvae were recovered from the lungs and mediastinal lymph nodes but not from the intestines. When the remaining 3 calves and 3 noninfected controls were inoculated orally with 15,000 O. radiatum larvae, patent infections developed only in the controls. At necropsy, 52 days after oral challenge, only the controls had worms in the intestinal tract.

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Hemoglobin types as an Indicator of Resistance to Haemonchus contortus Infection in Sheep. R. E. BRADLEY* and A. F. JILEK

A 2x2 factorial-designed experiment, with 2 foundations (120 Florida Native and 60 Rambouillet ewes) and 2 levels of resistance to Haemonchus contortus (high and low) was conducted over an 18-month period. Allotment of ewes to high- or low-resistance groups was on the basis of mean hemoglobin levels for individual ewes determined from blood samples collected every other month for 2 years immediately preceding the 18-month experimental period. Florida Native ewes had consistently higher hemoglobin concentrations and packed cell volumes than did Rambouillet ewes. Within breeds, the high-resistance ewes had greater values for both determinations than the low-resistance ewes. Breed differences were observed in mean H. contortus ova counts; Florida Native ewes had consistently lower ova counts, of which more than 90% were H. contortus. Electrophoretic analysis of blood samples for hemoglobin type revealed a higher incidence of hemoglobin type A in Florida Native ewes than in Rambouillet ewes. Resistance group differences within the Florida Native ewes approached significance at the 5% level and hemoglobin type had a highly significant correlation with hemoglobin levels and packed cell volumes. Florida Native ewes with hemoglobin type A or AB had higher mean hemoglobin levels and packed cell volumes than did ewes with hemoglobin type B. Ewes with type A hemoglobin may therefore be more resistant to infections with H. contortus than ewes with type B.

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Trichinella spiralis Infections in Neonatally Thymectomized Rats

R. W. GORE*, H.-J. BURGER and E. H. SADUN

It has been suggested that immunity in Trichinella spiralis infections may be due to a specific delayed hypersensitivity reaction. Experiments were designed to test whether neonatal thymectomy would alter the natural resistance of rats to infection with this parasite. In replicate experiments, over 100 rats were subjected to neonatal thymectomy or sham-operation within 24 hours after birth. The effectiveness of the operation was determined by the number of lymphocytes in the peripheral blood at 3 to 6 weeks of age. All the animals and non-operated controls were infected with T. spiralis at approximately 2 months of age with 10 or 20 larvae/gm body wt. In each experiment, groups of animals were killed 1 week, 2 weeks, and 6 weeks after infection and the parasite burdens were analyzed to evaluate the influence of thymectomy on the course of infection of rats with T. spiralis. Antibodies to T. spiralis in various groups were tested by the soluble antigen fluorescent antibody technique and by passive cutaneous anaphylactic tests. The results indicate that the production and persistence of either fluorescent or anaphylactic antibodies was not impaired by neonatal thymectomy.

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Cell-mediated Immunity in Rats Infected with Trichinella spiralis.

H. J. BÜRGER,* R. W. GORE and E. H. SADUN

Attempts were made to passively transfer immunity to Trichinella spiralis in rats by sensitized lymphoid cells. In two experiments involving a total of 138 inbred Fischer rats, immunity to normal recipients could be transferred by 10^7 to 10^9 mixed lymphoid cells of thymus, spleen, and lymph nodes from previously hyperinfected cell donors. In both experiments, 1 week after infection the number of worms recovered from the intestine was similar in the groups that received cells from immunized and non-immunized donors, as well as in rats which did not receive any cells. Conversely, two weeks after infection, significantly fewer adult worms were found in the "immune" cell recipients, and 5 weeks after infection, lower numbers of larvae were detected. Passive cutaneous anaphylactic antibodies occurred earlier in "immune" cell recipients. In both experiments they were present in most of the "immune" cell recipients 2 weeks after infection. No difference was observed in the levels of antibodies detected by the soluble antigen fluorescent antibody technique. These data support the hypothesis that lymphoid cells take part in the immune reaction(s) in T. spiralis infections in rats.

100

Transformation of Lymphocytes from Animals Sensitized to Trichinella spiralis.

CHARLES W. KIM*, MAHENDRA P. JAMUAR and L. D. HAMILTON

Guinea pigs were sensitized either once or 4x at 2-week intervals with T. spiralis antigen combined with complete adjuvant and skin-tested 7 or 56 days later. The skin reaction was typically delayed in animals sensitized only once; precipitating antibody was not detected although antibody was detected by passive cutaneous anaphylaxis (PCA). The skin-test site showed perivascular infiltration of exclusively mononuclear cells. The skin reaction was Arthus-like in animals repeatedly sensitized; both PCA and precipitating antibodies were detected. However, the skin-test site showed perivascular infiltration of mononuclear cells. Lymphocytes (1×10^8) from lymph nodes removed from both groups, inoculated with 0.1-0.5 ml of antigen, were incubated at 37° C under ~ 5% CO_2 for 5 days. Before incubation smears (1:50 Giemsa) from animals sensitized once showed that small lymphocytes predominated; in contrast in smears from repeatedly sensitized animals medium-sized lymphocytes predominated. Both groups had similar proportion of morphological transformation of lymphocytes to "blast-like" cells (10%) after incubation. The cells had large nuclei - some in mitoses - with highly vacuolated, enlarged cytoplasm. There was transformation of 0-2% of cells incubated without antigen. Evidently although repeated sensitizations stimulate antibody production and macroscopically interfere with delayed response, they do not increase the number of lymphocytes commited to transform into "blast-like" cells.

(Supported by U.S. AEC.)

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Isolation of Naegleria gruberi from Nasal Swab of a Healthy Individual,

G. HEALY,* J. SHUMAKER, F. PAGE, D. ENGLISH, and M. SCHULTZ

Naegleria gruberi, a free-living ameba, has been implicated as the causative organism in fatal cases of meningoencephalitis and several other species of free-living hartmannelids have been isolated from human pharyngeal swabs. This report deals with the recovery of N. gruberi from the nasal swab of one of 155 children enrolled in a swimming program at two lakes near Richmond, Virginia. Nasal swabs taken from children before and after swimming were placed in Hank's balanced salt solution, and within eight hours, the fluid inoculated onto 1.5% Bacto Difco agar plates seeded with Aerobacter aerogenes. Plates were incubated at 25° C and 37° C. Paired specimens from 40 children were also inoculated into monkey kidney tissue culture tubes and incubated at 37° C.

Amebae were recovered in a 25° C agar plate incubate from the post-swimming nasal swab of a seven-year-old male student. The child had no antecedent or subsequent upper respiratory tract, sinus, or ear infection. Studies of the morphologic characters of the amebae isolate showed them to be N. gruberi as evidenced by type of cyst, development of biflagellated forms upon exposure to hypotonic media, and the presence of

polar masses and interzonal bodies in mitotic figures of reproducing forms.

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A Plasmodium similar to P. mexicanum in Arizona lizards. SAM R. TELFORD, JR.*

Examination of blood smears from 149 Sceloporus jarrovii Cope, collected in the Chiricahua and Graham mountains, Arizona revealed the presence of a Plasmodium in 42 lizards (28%). It is not considered to be P. mexicanum Thompson & Huff, although it is similar in some morphological and biological characteristics.

The infection rate varied altitudinally: 54% (N=57) between 6000 and 7900 feet; 26% (N=35) from 8000 to 8900 feet; and 6% (N=36) between 9000 and 9900 feet. Maximum elevation at which an infected lizard was found is 9200 feet, although 22 lizards collected at 9400 feet and 21 from 10,700 feet were examined.

In the sample collected between 6000 and 7900 feet, the infection rate in June (N=32) was 50%, and in September to November (N=24), 63%. Asexual parasites were present in one-third of the infections in each period, with two-thirds of the infections being comprised only of gametocytes. The levels of parasitemia were approximately the same in each sampling period.

Haemogregarine parasites also present showed no similar altitudinal restriction: infection rates were 42% between 6000 and 7900 feet; 17% from 8000 to 8900 feet; 28% from 9000 to 9900 feet; and 48% in those hosts collected at 10,700 feet.

103

Repetitive Transmission of P. berghei yoelii in an A. stephensi-Mouse System, LIONEL T. RICHARD, MAURICE E. KING* and ALAN M. SHEFNER

A method for providing weekly sporozoite-induced infections of P. berghei yoelii was developed as part of an antimalaria screening program. Two blood passages at 6 and 4 day intervals are made after an initial sporozoite infection in Ha/ICR mice. Unanesthetized donor mice are used for the infective blood meal of female Anopheles stephensi on the fourth day after the second blood passage. Mosquitoes are then maintained on 10% sucrose while being held in unlighted incubators at 24°C and 90% relative humidity. Sporozoite infections have been obtained with mosquitoes incubated from 12 through 17 days, however 14 days was selected for optimal infection rate in mice with minimal mosquito mortality. For the infection, mosquitoes are triturated with saline in glass mortars at 0°C and the mixture centrifuged for 5 minutes at 1000 RPM. Each mouse is injected intraperitoneally with 0.2 ml of the supernatant liquid containing the equivalent of 3 mosquitoes. Patent infections are obtained by 3 days after injection and essentially all mice are patent after 6 days. This procedure has been used for weekly infections for 18 months and for the past 6 months in testing drugs for antimalarial activity.
(Supported by U.S. Army Contract DA-49-193-MD-3027.)

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Characteristics of Sporozoite-Induced Plasmodium berghei yoelii Infections in Mice after 46 to 72 Hours Incubation. MORRIS D. SCHNEIDER*, MAURICE E. KING, LIONEL T. RICHARD, NELLA SEAL, and ALAN M. SHEFNER

Ha/ICR mice had been intraperitoneally (IP) injected 3 Anopheles stephensi infected with P. berghei yoelii. Two experiments were concerned with determination by bioassay IP in mice of duration of prepatency, maturation and reproduction of primary exoerythrocytic (EE) schizogony stage in livers taken from nontreated mice exsanguinated 46 to 72 hr after sporozoite induction of the malaria. The extent of dissemination and relative growth of parasites in other host tissues were also determined. The primary EE stage in liver was found infective for mice by 46 hr. Bioassay showed an estimated burden of <1 infectious malarial units (IMU) per liver per mouse. By 48 hr incubation, infective EE malaria multiplied to 1.73×10^2 IMU and by 70 to 72 hr reproduced to 7.05×10^4 per liver per mouse. Results of assays of erythrocytes and various organs taken from mice after 46 to 48 hr, and 70 to 72 hr were as follows: (1) in RBC, $>1.73 \times 10^2$ and $>1.65 \times 10^5$ IMU, respectively; (2) in spleen, 4.7 and $>2.48 \times 10^2$; (3) kidney(s) were still in noninfective phase by 46 to 48 hr, but showed 1.85×10^2 IMU by 70 to 72 hr. Primaquine prophylactically injected in mice with anopheline-induced malaria acted as strict schizontocide agent in EE stage in liver. DDS injected in sporozoite infected mice was inactive as causal prophylactic agent.

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Schizogonic Cycles of Leucocytozoon dubreuili and L. fringillinarum, R.A. KHAN*

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Ornithophilic simuliids were collected from American robins (Turdus migratorius) and grackles (Quiscalus quiscula) which harboured gametocytes of L. dubreuili and L. fringillinarum respectively. Sporozoites of L. dubreuili and L. fringillinarum were injected into uninfected robins and grackles respectively and in addition, cross-transfer experiments were conducted. Blood smears were prepared daily from the birds. Hepatic biopsies were performed on some of the injected birds and these as well as several others were killed at intervals following injection. Cross-transfer experiments established the specificity of the respective species of Leucocytozoon. Schizonts of L. dubreuili and L. fringillinarum were observed in hepatic and renal parenchymal cells of the infected birds. The course of development and corresponding times were noted. Sporozoites of L. dubreuili were observed in impression smears of the liver of some birds up to 11 days after their inoculation. The significance of this observation is discussed.
(Supported by a scholarship from the Province of Ontario and M.R.C. grant MT 2998 to Dr. A.M. Fallis.)

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Simulium innocens a new vector of Leucocytozoon simondi in Canada geese (Diptera: Simuliidae), I. BARRY TARSHIS

High losses of Canada geese goslings due to Leucocytozoon simondi have occurred at the Seney National Wildlife Refuge, Seney, Michigan every four years. Bird exposure studies made at the Seney Refuge during May and June between 1964 and 1968 prior to and at the time the goslings are becoming infected and dying of Leucocytozoon show that three species of ornithophilic black flies are present: Cnephia invenusta, C. taeniatifrons and Simulium innocens. Engorged C. invenusta and C. taeniatifrons recovered from field exposed Leucocytozoon infected mallards and Canada goslings, held from 2½ to 6 days and inoculated intraperitoneally, intravenously and intramuscularly into parasite-free mallards failed to produce the infection in these birds. Engorged S. innocens recovered from field exposed Leucocytozoon infected mallards and Canada goslings, held 4 and 5 days and inoculated intravenously into two parasite-free mallards produced infection with Leucocytozoon. Round stage gametocytes were observed in the red blood cells in 10 days in one bird and 11 days in the second bird following inoculation. Simulium rugglesi a long suspected vector of Leucocytozoon in Canada goslings at the Seney Refuge does not occur on the Refuge until after the goslings have become infected and/or died from Leucocytozoon.

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Transfer of a hemogregarine from Boa constrictor to a lizard Anolis carolinensis by a mosquito vector, THEODORE BOODEN, JOWETT CHAO and GORDON H. BALL*

Previous investigations in this laboratory have shown that a hemogregarine, Hepatozoon rarefaciens can be transferred from an indigo snake to snakes of two other genera by feeding infected mosquito vectors to the snakes. We now report the transfer of a different species of Hepatozoon from another kind of snake, Boa constrictor to a lizard, Anolis carolinensis by the same route. In Culex tarsalis, which had bitten a B. constrictor with hemogregarine gametocytes in circulating erythrocytes, the mosquitoes had oocysts and sporocysts with sporozoites in the hemocoel 20 days later. These mosquitoes were fed to 2 Anolis, negative for blood sporozoa for 4 months. One month after ingesting the parasitized mosquitoes, both Anolis showed hemogregarines in the peripheral blood. At this time, C. pipiens was fed on one of the infected Anolis. Sporogonic stages identical to those found in C. tarsalis were present in the hemocoele 3 weeks later. Anolis fed infected C. pipiens were positive after 4 weeks. To our knowledge, this is the first demonstration that a hemogregarine from a member of the Serpentes can infect a lacertilian. It provides additional evidence of the capacity of some species of hemogregarines to develop in very different kinds of reptilian hosts.
(Supported by NSF Grant 7069 and Univ. Calif. Grant 254).

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Pathway and Timing of Invasion of Sporozoites of Eimeria stiedae (Lindemann, 1865), Robert L. Slater*, M. A. Quisenberry and P. R. Fitzgerald

The route of invasion of the sporozoites of E stiedae between the duodenum and the bile ducts was investigated. Domestic rabbits were inoculated with sporulated oocysts and killed at various intervals between 5 and 120 hr post-inoculation. Free sporozoites were present in smears and sections of mesenteric lymph node (16-120 hr), and within the sinusoids(72 hr) and periportal regions of the liver(72 hr) Intracellular sporozoites were observed in the duodenal mucosa(5-9 hr), within lymphatic monocytes of smears or sections of the mesenteric lymph node(16-120 hr) and portal blood(72-120 hr) or in the liver(72 hr), and within the bile duct epithelium(72 hr). The demonstration of sporozoites of E. stiedae within monocytes of the portal blood and portal tributaries of the liver supports the hypothesis that the route of infection of the liver is via the portal blood from the mesenteric lymph node. (Supported by NIH Grant FR-00410.)

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Incidence of Several Species of Chicken Coccidia in Some Poultry Raising Areas of the United States, R. L. KENNETT, JR.*, G. T. WANG and S. KANTOR

Though there is considerable information on comparative incidence of Eimeria species in chickens for the U.S.A. (Avian Dis. XII (4): 730-754, 1968), diagnosis is generally unconfirmed by subsequent laboratory passage. The following incidences were confirmed by two or more laboratory passages for samples from 41 flocks from nine states: E. acervulina (and/or mivati) 93%, E. tenella 49%, E. brunetti 24% and E. necatrix 20%. In 19 flocks, incidence of E. maxima was 32%. Although some of the flocks were considered to be clinically affected, in others coccidiosis was not considered the primary cause of morbidity. Disregarding possible E. mivati, 12% contained one species; 46% contained two species; 37% contained three species; 5% contained four species, and none contained all the species simultaneously.

The states from which flocks were sampled were: Alabama (9), Arkansas (3), California (2), Delaware (1), Florida (2), Georgia (10), Maryland (3), New Jersey (2), and New York (9).

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Role of a Hyperparasite (Urosporidium) in the Dispersal of Microphallid Metacercariae from the Blue Crab, JOHN A. COUCH* and MARTIN W. NEWMAN

Urosporidium crescens DeTurk, 1940, a haplosporidan parasite, and its host Microphallus sp. (metacercariae) are found in blue crabs (Callinectes sapidus) from the Atlantic coasts of the southeastern United States. As many as 45% of blue crabs in certain locales are infected with the metacercaria and its parasite.

The cercariae are reported to enter the blue crab via the efferent vein of the crab's gills, then make their way in the hemolymph to the heart, hepatopancreas, and body muscles of the host where they encyst. Often metacercariae, lightly to heavily parasitized by Urosporidium crescens, are found on the gill surface, between gill lamellae, in the hepatopancreas, and in the skeletal muscles of the crab. In heavily parasitized metacercariae the parenchyma and organ tissues are completely replaced by plasmodia, sporocysts, and spores of Urosporidium. The metacercariae eventually become filled with Urosporidium stages and appear darkly pigmented. Heavily parasitized metacercariae are hypersensitive to the slightest mechanical pressure and rupture very easily. Therefore, the hyperparasite acts as a limiting factor in the dispersal of the trematode to its definitive bird host, and may aid the crab host by destroying the trematode. Stages of Urosporidium have not been found to infect any tissues of the blue crab.

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The trematode, Aponurus sp., and its definitive host the deepsea smelt, Leuroglossus stilbius, E.R. NOBLE* and J.D.ORIAS

Mid-water fishes of the eastern Pacific rarely harbor adult digenetic trematodes. Leuroglossus stilbius (Family Bathylagidae) is a mesopelagic fish ranging from the Bering Sea to Columbia. A new species of the trematode genus Aponurus is commonly found to inhabit the stomach of these fishes living near the Santa Barbara Channel Islands, but apparently not elsewhere in the geographic range of the host. Intermediate hosts for this trematode have not been found, although numerous associated copepods and other invertebrates have been examined. L. stilbius migrates to the surface each night and possibly acquires cercariae in surface waters. In the daytime it may descend to the bottom (500-600 meters in the Santa Barbara Basin) and might become infected with cercariae from bottom-dwelling arthropods or mollusks. Adult preserved Aponurus are about 1 mm long, and are characterized by a genital opening located near the anterior sucker, absence of a genital atrium, tandem testes, and eggs about 20 X 50 microns. (Supported by NSF Grant GB 6356.)

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Fertilization of Paragonimus westermani in Experimental Animals
P. C. Fan* and C. H. Chiang

Thirty seven (37) puppies and 23 kittens were infected, each with one metacercaria of P. westermani, then sacrificed, except 15 puppies and 3 kittens, at interval from 76 to 116 days after infection. The main purpose is to determine the fact that self-fertilization is also a factor related to sexual maturity of P. westermani. The data are summarized as follows: 1) The worm recovery rate was 61.90% (26/42); it is significantly higher in kittens (70% or 14/20) than that in puppies (54.54% or 12/22). 2) The number of worms, 6, 7 and 12 respectively was found in the pleural cavity, lung-lesions and wormcysts of the lungs in experimental animals. 3) There were 17 mature worms, 11 were in wormcysts, 4 in lung-lesions and 2 in pleural cavity. 4) Miracidium was found actively moving within the eggs from the above worms and/or freely swimming in the water 19-27 days after culture, proving that they were fertile and mature. In general : The kitten is a more favourable host than a puppy; the formation of wormcysts can be developed by stimulation of a worm; mature worms can be found not only in the wormcysts, but also in the lung-lesions and pleural cavity; a single worm can develop to sexual maturity without a mate in the same wormcyst; self-fertilization is also a factor related to sexual maturity of P. westermani in experimental animals; and the eggs produced by a single mature worm have been proved to be fertile. (Supported by CMB grant 66914)

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Cephalopods as Intermediate Hosts for Larval Didymozoids. F.G. HOCHBERG, JR.

Larval didymozoids representing a single species of the genus Monilicaecum Yamaguti, 1942, have been recovered from cephalopods in the North Pacific Ocean. The immature trematodes are restricted to the caeca and intestines, ie. the non-keratinized portions of the digestive tract. Several squid when examined had actively excysting metacercarial stages in the stomach. Genera of hosts commonly infected are the mid-water forms Pterygioteuthis, Pyroteuthis, Abraliopsis, and Symplectoteuthis, and the benthic-oriented Loliolopsis. In the life-cycle as illustrated by Nikolaeva, 1965, small fish serve as third alternate or reservoir hosts. Squid functioning at the same trophic level can also become infected and as indicated in this study they also serve as reservoir hosts in the life-cycle of the didymozoids. Thus, both groups are primary carnivors which feed on copepods and other small crustaceans at the time of infection. Madhavi, 1968, reported a Monilicaecum-like metacercaria in Paracalanus aculeatus. The distribution of this calanoid copepod corresponds rather closely to that observed for the trematode infection in squid. It is suggested that paratenic hosts are essential for transfer of these parasites through the food-chain to progressively larger animals and eventually to final hosts, as yet unknown.

(Supported by PHS Trainee Grants 5 TI-GM 990-02 and 5 T01 AI00327-02)

114

Re-Investigation of Trichinella spiralis Life Cycle, WIESLAW J. KOZEK.

Light and electron microscopic studies were conducted to elucidate the aspects of T. spiralis life cycle which are still disputed, namely: the number of molts, where and when they occur. Ultrastructural examination revealed that the young larvae do not molt in the uterus of mature females. Similarly, both light and electron microscopic examination of larvae fixed in situ, or teased from the diaphragm of rats, which were sacrificed daily from 6th to 22nd day post infection, failed to reveal molting during the intramuscular phase of development. Examination of synchronously developing populations of T. spiralis, obtained by injecting infective, excysted larvae directly into the duodenum of rats, disclosed that both males and females undergo 4 molts during the intestinal phase of development. Molting could be demonstrated at both light and ultrastructural levels. The peak of molting periods occurred at about 9, 13, 18, and 25 hours for the males, and about 10, 15, 21, and 28 hours for the females. Inseminated females were observed as early as 30 hours after the larvae were injected into the duodenum. The results of this study support the observations of other investigators who reported that T. spiralis molts 4 times in the intestine, and indicate that the life cycle of T. spiralis has the same five-stages-four-molts pattern which is observed during the development of phasmid and free-living nematodes.

(Supported by grants AI-00002 and AI-04919 from the N.I.H., U.S. Public Health Service.)

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The Role of Ingested Hemoglobin in the Nutrition of Schistosoma mansoni.

R. A. ZUSSMAN*, P. M. BAUMAN and J. C. PETRUSKA

In vivo tracer studies in mice with adult S. mansoni prove that ingested host red blood cells are involved in schistosome nutrition. Homologous reticulocytes, labeled with tritiated L-leucine, were injected into the circulation of infected mice. Four days post-injection, the animals were killed and the schistosomes were recovered by perfusion. The harvested worms were examined for radioactivity by direct counting and radioautography. These techniques demonstrate that L-leucine, bound in labeled hemoglobin, becomes extensively distributed and incorporated in worm tissue. Female worms become more strongly labeled than males, supporting the work of other investigators that females are metabolically more active than males. Our studies support the contention that "schistosome hemoglobin protease" is involved in worm nutrition and that inhibitors of this enzyme, if found, could lead to therapeutic use by specifically interfering with normal schistosome nutrition.

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Effects of NaCl and Glucose on Longevity of Miracidia and Cercariae of Schistosoma mansoni, H. L. ASCH* and D. V. MOORE

As a prelude to metabolic studies media were sought which would provide optimum conditions for survival of each of the fee-living stages of S. mansoni. Miracidia were individually examined every hour for 8 hours after emergence and cercariae for 10 hours. Motility was the criterion of viability. In experiments with 200 miracidia optimal NaCl concentration was 0.01M. Glucose added to this solution to a final concentration of 0.03M was found to effect the longest survival. 225 cercariae were similarly observed and a solution of 0.01M NaCl and 0.003M glucose yielded maximum longevity. (Supported in part by PHS Training Grant No. 5T01 AI00142, NIAID.)

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Rate of Destruction of Schistosoma mansoni Eggs in the Tissues of Mice.

LOREN A. ANDERSON* and ALLEN W. CHEEVER

Mice infected with S. mansoni were treated with stibophen 7 weeks or 24 weeks after infection. One month after early treatment, 40% of eggs present at the time of treatment remained in the tissues, 50% had been destroyed in the tissues and 10% passed in the feces.

Mice treated 24 weeks after infection also destroyed eggs rapidly in the first few months after treatment. Thereafter the rate of egg destruction slowed considerably, and the number of eggs remaining was nearly the same 36 weeks and 56 weeks after treatment.

It seems likely that two "pools" of eggs exist. A portion of the recently laid eggs are rapidly destroyed. Eggs not destroyed during this period enter a "pool" in which egg destruction proceeds more slowly. These eggs are presumably those surrounded by dense fibrous tissue. It is also possible that the decline in the rate of destruction of eggs after treatment is partly related to the lack of antigenic stimulus, i.e. the antigenic stimulus of eggs and dead worms may accelerate egg destruction in the first months after treatment.

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Effect of Schistosoma mansoni on Heart Rate and Oxygen Consumption of Biomphalaria glabrata, CARL S. HACKER

The determination of the heart rate of B. glabrata is possible by direct observation of the heart through the shell of the intact snail. Snails infected with S. mansoni were found to have a heart rate greater than that of uninfected controls. By examining groups of exposed and unexposed snails at weekly intervals, it was shown that the mean heart rates of exposed and control groups did not differ until after the 14th day following exposure. No differential effect could be detected between snails which had been exposed to either 5 miracidia each or 25 miracidia each. Manometrically determined rates of oxygen consumption by snails removed from their shells supported the observations on heart rate for infected snails. The above observations can be related to changes in the polysaccharide content of B. glabrata subsequent to infection and also to other physiological alterations. (Supported by NIH Grants 5 TI AI00106 and AI01384.)

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Ultrastructure of the Cercarienhullen Reaktion of Schistosoma mansoni, W. M. Kemp*

The fine "extra-cuticular film" of Kruidenier and Stirewalt (1955) on the cercariae of Schistosoma mansoni and its role in the Cercarienhullen Reaktion is confirmed. This "extra-cuticular film" is resolved by electron microscopy to be a pronounced hirsute coat, which appears to intimately associate with factors in homologous anti-serum to form the Cercarienhullen sheath. Light and electron microscope histochemistry suggest the presence of glycoproteins in the hirsute coat. (Supported in part by NIH Grant GM-669 and NSF Grant GB 7938.)

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Cercariophagic Activity of Guppy Fish (Lebistes reticulata) Determined with Radioactive Cercariae, WILDA B. KNIGHT*, L. S. RITCHIE, and J. CHIRIBOGA

Quantitations of cercariophagic activity of guppies were made using cercariae labeled with radioselenium (^{75}Se). Most guppies, regardless of sex or maturity, became radioactive after being fed cercariae. Individual differences were marked and heavily gravid females became radioactive only infrequently. The intestine had about half as much activity as the rest of the body 24 hours after feeding, indicating that ingestion had occurred and that products of digestion had probably been absorbed. Since some fish did not take up radioactivity, it appears unlikely that cercariae penetrated tissues of the fish. When the number of cercariae per unit of volume was varied by feeding the same number in 250, 1800, and 4000 ml of water, a gradient of radioactivity occurred in the fish. A corresponding gradient did not occur when the number of cercariae was varied in a constant volume of water. Guppies that were fed normal cercariae repeatedly (familiarized) prior to giving them labeled cercariae became radioactive more quickly than fish that had not encountered cercariae previously. Guppies took up radioactivity more slowly in darkness than in the light. After one hour fish in total darkness were essentially normal, and after four hours the fish in the light were five to six times as active. This difference was less after 24 hours. The findings indicate that predation occurred rather than chance ingestion coincident with respiration.

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Scanning Electron Microscopy of Schistosome Cercariae, Schistosomules, and CHR Reactions, P. L. MORIEARTY* and R. M. LEWERT

Scanning electron microscopy was performed on the following schistosome specimens: 1) cercariae of laboratory strains of Schistosoma mansoni and Schistosomatium douthitti; 2) S. mansoni schistosomules collected from mouse skin 24 hours after penetration; 3) cercariae and schistosomules following incubation in normal and immune monkey sera. All specimens were fixed in gluteraldehyde and coated with a thin layer of gold before observation. Photomicrographs, taken on an experimental scanning electron microscope developed at the University of Chicago by A. V. Crewe and associates, are comparable to those produced on commercial scanning electron microscopes. Details of cercarial structure, including penetration spines and

sensory papillae, were visualized. Classical cercarienhullen (CHR) reactions were observed in S. mansoni cercariae, and details of the structure of the CHR envelope have provided some indications of its possible origin.
(Supported in part by USPHS Training Grant AI00331, and Grant AI00884.)

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Egg-shell Histochemistry in Four Species of Digenetic Trematodes, PAUL M. NOLLEN.

Histochemical tests for precursors and enzymes involved in egg-shell formation were carried out on Haematoloechus medioplexus, Megalodiscus temperatus, Gorgoderina attenuata, and Philophthalmus megalurus. Of these only the eggs of H. medioplexus appear tanned in lvivng specimens. Burton (1963, J. Exp. Zool. 154: 247-257) investigated the egg-shell chemistry of this species and found a quinone tanning pathway. The histochemical reactions of H. medioplexus were compared to those in the other three species, which have egg-shells that are thin, leastic, and do not appear to be tanned. Histochemical tests for tyrosine and phenols indicate that precursors for quinone tanning are present in all 4 species investigated. The enzyme tyrosinase was demonstrated only in H. medioplexus and P. megalurus by the catechol method. Using Becker's dopa-oxidase technique, this enzyme was again found in both species, but when tyrosine was used as a substrate only H. medioplexus gave a positive reaction. These results indicate that various means of egg-shell formation may be found among the trematodes. G. attenuata and M. temperatus seem to lack tyrosinase and must have other means for binding the shell. Madhavi (1968, Exp. Parasit. 23: 392-397) could not demonstrate tyrosinase histochemically in Diplodiscus medhrai and postulated a keratin-type binding. Further investigations are being carried out on the species involved in this study for S-S binding. P. megalurus has an enzyme that will oxidize dihydroxyphenols but not monohydroxyphenols. This indicates two enzymes may be involved in the sclerotization process in digenetic trematodes and one is missing in this species. (supported by a grant from the Research Council, Western Illinois University)

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Fractionation of Particle-associated Antigens and Enzymes of Trichinella spiralis by Isoelectrofocusing. DICKSON D. DESPOMMIER

Functional antigens, able to elicit resistance in mice, are associated with the stichosome cell granule fraction of homogenates of whole Trichinella spiralis larvae (Despommier and Müller, 2nd Internat. Confer. Trichinellosis, Wraclaw, Poland, June, 1969). These antigens can be released from the granule fraction by treatment with Triton X-100. These solublized proteins were applied to an electrofocusing column in an attempt to separate and identify them. Acid phosphatase and N-acetyl-B-glucosaminidase were also associated with this granule fraction. Therefore enzyme assays, as well as Ouchterlony analysis, were used as markers. When the pH gradient used was 3-10, two separate peaks of N-acetyl-B-glucosaminidase (IP 4.10 and 4.45) and three to five peaks of acid phosphatase (IP 3.85-4.38 and 4.80) were obtained. Ouchterlony analysis revealed 5 precipitin systems with IP of 4.15-6.25. This technique permits the full recovery of the sample and enzyme assays can be directly carried out in soluble phase.
(Supported by USPHS Post-doctoral Fellowship 1-F2-A1-31 and NIH Grant A1-04842-05).

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The Use of a Soluble Antigen Fluorescent-Antibody Test to Diagnose Trichinella spiralis in Swine, ROBERT S. ISENSTEIN*

A soluble antigen fluorescent-antibody test was used to test for the presence of Trichinella spiralis in swine. Serum was judged positive for T. spiralis antibody as early as 12 days following infection with 5,000 larvae. Increasing the size of the infection progressively delayed the onset of antibody detection. Sera from animals judged positive remained positive continuously to at least 100 days following infection. Sera from 17 of 18 pigs infected with more than 5,000 larvae produced tests judged positive and serum from one pig gave a marginal test. No sera from uninfected animals or animals infected with Ascaris suum gave positive tests although 2 of 3 sera from animals infected with Stephanurus dentatus gave marginal tests.

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Further Characterization of Haemonchus contortus Exsheathing Fluids, N. H. OZEROL* and P. H. SILVERMAN

Desalted metabolites from 3rd and 4-5th stage of H. contortus larvae were fractionated by Sephadex G-200 under standardized conditions. Two major fractions were obtained and their optical absorbancies were examined at 260 and 280 mμ. First peak of each metabolite, which was tested for protection in a field trial, was recovered and characterized by gel diffusion, physical and biochemical methods. Metabolites of both larval stages showed some serological activity with a resistant sheep serum. However, these activities were restricted to first peak of each metabolite.

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These metabolites were further fractionated by Bio-Gel A-1,5m under calibrated conditions and resulted in 3 major peaks. Their molecular weights, which were estimated by gel filtration, were approximately 650,000, 240,000 and 35,000 respectively. Polyacrylamide gel and cellulose acetate paper electrophoresis were utilized for further resolution of these fractions. Infrared spectrum of these metabolites yielded some preliminary information about their functional group. Their hydrolysis resulted in major amino acids. (Supported in part by USPH Grant AI 05910.)

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Anterior Nervous System of Macracanthorhynchus hirudinaceus

T. T. Dunagan & D. M. Miller*

Morphologically the nervous system of M. hirudinaceus differs significantly from other helminths. It especially lacks the organizational complexity found in either cestodes or nematodes. The anterior nervous system consists of: 1. Cerebral ganglion of only 84-88 cells within the proboscis receptacle. 2. Only a few (3 pair, 2 single) nerves leave this gnaglion and at least one is encased in a muscular sheath. 3. A pair of external sensory bulbs and a single apical sensory organ. Microelectrodes can be inserted into the large ganglion cells and by established techniques their exact position later determined. Using different types of microelectrodes, the following have been observed: 1. Very slow muscle potentials from the body musculature. 2. Numerous potentials of various magnitudes from specific cells in the cerebral ganglion. Certain of these potentials occur at regular time intervals. 3. Generator potentials triggering certain depolarizations which are followed approximately 0.4 seconds later by muscle potentials.
(Supported in part by a grant from the Office of Research and Projects, Graduate School, S.I.U.)

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Scanning Electron Microscopy - Observations on a Selected Group of Parasites, P. A. MADDEN* and J. M. VETTERLING

The scanning electron microscope is a relatively new instrument and has been commercially available for only the past few years. It differs from the conventional transmission electron microscope in both principal and design. Because of the resolution and depth impossible to achieve with other microscopes, research workers are able to obtain greater morphological detail from gross specimens.

We examined a group of selected parasites representing those studied at the Beltsville Parasitological Laboratory. Included in the presentation are micrographs of: (1) nematodes, both en face and lateral views. The en face views show the mouth, the lip surfaces with papillae and amphids, and the arrangement of the denticles in relation to the internal and external lip surfaces. The lateral views show cuticular lesions in detail and some microbial organisms within; (2) a fluke, in ventral position showing the ventral sucker and spines; (3) an appendage of a mite including a high magnification micrograph of a peduncle; (4) dorsal and ventral views of tick nymphs showing the mouth parts in detail in the ventral view and the setal structure in the dorsal view; (5) bovine blood parasitized with Anaplasma, demonstrating the limitations of the scanning electron microscope, and with Eperythrozoa showing the situation of these extracellular parasites; and (6) coccidia, showing the surface structure of oocyst hulls and free sporozoites.

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Effect of Temperature on the Feeding Mechanisms of Nymphal Hyalomma aegyptium Ticks.

G.K. SWEATMAN* and J.D. GREGSON

The important factor controlling engorgement of subadult Hyalomma aegyptium ticks on poikilothermic hosts was temperature. The threshold temperature for complete engorgement was 20 C below which both larvae and nymphs fed only occasionally and ultimately died before completing engorgement while still attached to the host. Between 20 and 30 C there was a highly significant negative correlation between engorgement period, as measured in days, and temperature. For each drop of 1 C the larval and nymphal engorgement periods increased by 1 1/2 and 3 1/4 days respectively. The daily larval drop from homiotherms, with skin temperatures between 32 and 40 C, showed no correlation between the engorgement period and skin temperature. With larvae on poikilotherms at 30 C and above there was also no relationship, but with nymphs a small correlation was apparent.
The conductivity of a small current passed through feeding nymphal H. aegyptium at 15, 20, 25, 30 and 35 C was measured on an oscilloscope and changes in the pharyngeal sucking action and the salivary secretion were recorded on film. Quantitative differences in feeding rate were the most conspicious changes associated with different temperatures.
(Supported by the United States Air Force under Contract F61052-68-C-0059.)

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Amino Acids in the Egg Shell of Rhipicephalus sanguineus. Benedict J. Jaskoski* and Veronica L. Butler.

Ticks as well as other parasites produce many hundreds of eggs. By defining the chemical constituents of the egg shell it may be possible to develop control measures that would prevent the formation or hatching of the eggs. Basic to an understanding of the chemistry of the egg shell is a determination of the amino acids of which it is composed. Lipids were extracted from the egg

shells of the common dog tick, Rhipicephalus sanguineus, with ether and the protein fraction was hydrolyzed in 6_N HCL. Thin-layer ascending chromatography was used to determine the amino acids present in the acid hydrolysate. Both one and two dimensional chromatograms were run using N-butanol: acetic acid: water (8:2:2) for the first dimension and N-propanol: water (7:3) for the second dimension. Twelve amino acids were found. Most abundant are lysine, glycine, alanine, threonine, tyrosine, glutamic acid, and valine. Arginine, leucine, serine, and aspartic acid are present in smaller amounts followed by isoleucine. The presence of chitin was indicated by the chitosan test of Campbell (1929. Ann. Ent. Soc. Am. 22:401-426). (Supported in part by Research Grants-in-Aid from Loyola University and the Illinois Academy of Sciences.)

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An Electron Microscope Study of Entamoeba histolytica Trophozoites in vivo,
J. H. MILLER*, R. H. GILMAN and V. M. VILLAREJOS

Observations of biopsy specimens from patients with amebiasis in Malaysia have shown marked morphological differences in the plasmalemma, vacuolar apparatus and endoplasmic reticulum from trophozoites observed in vitro. Two types of plasmalemma extensions are seen in addition to blunt pseudopodia: (1) clumps of very fine pseudopodia, resembling filipodia, and; (2) small oval vesicular structures which appear "to bud" in an apocrine-like manner. Profiles of the endoplasmic reticulum are increased and stacking may be seen occasionally. Numerous small vesicles are present in the cytoplasm and at the plasmalemma where they may be related to the extensions mentioned previously. (Supported, in part, by NIH Grant AI-02347 and U.S. Army MRDC Contract DADA-17-68-C-8023)

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The Fine Structure of Merozoites and Resulting Gametocytes of Leucocytozoon simondi,
S.S. DESSER* and J.R. BAKER.

Merozoites released from megaloschizonts are bounded by a unit membrane interrupted by an apical pore through which empty dense paired organelles. A single mitochondrion and nucleus lie within a relatively membrane-free, ribosome filled cytoplasm. These merozoites apparently penetrate and develop exclusively in leucocytes, most frequently lymphocytes. The parasite is invested by host membrane within the white cell. Dense granules accumulate between the parasite membrane and that of the host to form a third thickened membrane-like layer, so that in the mature gametocyte a 13 layered complex bounds the parasite. Merozoites of L. simondi do not possess a feeding oriface or "cytostome" and intake of nutrients prior to formation of the thickened membrane complex is probably through diffusion. Host cytoplasm is engulfed by the maturing gametocyte by phagotrophy. The characteristic elongation of infected leucocytes begins soon after entry of the merozoite. The host centrioles are apparently induced by the parasite to produce large numbers of microtubules which stretch the infected cells laterally to form the "cytoplasmic wings". The development of merozoites from hepatic schizonts in erythrocytes to form round gametocytes will be compared to the above. (Supported in part by Medical Research Council grant MA 3686.)

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Cytochemistry of the Pellicle of Trypanosoma brucei and its Role in Cell Shape,
K.A. WRIGHT* and H. HALES.

A cell coat ~ 100 Å thick, consisting of an apparently amorphous material has been found associated with the outer surface of bloodstream forms of Trypanosoma brucei. Techniques for the cytochemical identification of polysaccharides by electron microscopy (i.e. modifications of the PAS-silver method, +ve charged iron colloid staining, and ruthenium red staining) indicate that polysaccharides occur in the cell coat immediately outside the cell membrane. These polysaccharides are masked from the external medium by proteins which can be removed by trypsin digestion. Treatment with trypsin drastically alters the animals sensitivity to osmotic distortion, indicating the role of the cell coat in maintaining the animal's morphology. The cell coat has been found to be resistant to treatment with EDTA and lysozyme although the animals are sensitive to the absence of Ca^{++} and Mg^{++} in calcium-magnesium free salt solutions.

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Fine Structure of Cephalic Gland Cells of Cryptocotyle lingua Cercariae,
P. L. KRUPA*, A. K. BAL and G. H. COUSINEAU

Membrane-bounded secretory granules or droplets, produced by cephalic (penetration) gland cells of Cryptocotyle lingua cercariae, were studied by electron microscopy. Rough endoplasmic reticulum and Golgi bodies appear to take part in granule production by way of a sequence of morphologically discernible stages. Electron lucent Golgi vacuoles may condense into presumptive granules containing an eccentric dense core. The dense core, appearing as a network of electron opaque fibers, is absent from granules in transit toward more apical portions of the cephalic gland apparatus. In a later developmental stage, dense spheroids (about 200 Å in diameter) appear to be emitted from the secretory granules. An amorphous patchlike structure, of slightly greater density than the rest of

the matrix, also occurs in secretion granules at about this stage in their maturation. The presence of such focally perceptible intragranular substructures infers that a complex heterogenous secretory product is manufactured by these gland cells. Microtubules, located along the inner margin of the plasma membrane of both gland cells and ducts, may serve as cytoskeletal and channeling devices in the ducts.
(Supported in part by The National Research Council of Canada Grant A-3624 to Dr. Cousineau, and by The City College of New York Research Foundation.)

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Morphology of Post-Embryonic Stages of the Tapeworm Hymenolepis citelli. WILLIAM K. COLLIN*

Post-embryonic stages of H. citelli were reared in the common flour beetle, Tribolium confusum at 30 C. Developmental stages at three and five days post-infection were dissected from the beetles and processed for electron microscopy. The outer surface of the cestode, its association with the beetle hemocytes, and some of the cestode cell types were studied.

The outer surface is composed of numerous thin microvillar structures which originate from a lower amorphous zone. The microvilli do not resemble those of the adult.

Beetle hemocytes often adhere to the microvillar region. The microvilli may be directed toward the hemocyte and in some cases they may pass through the plasma membrane. Destruction of the hemocytes is evident as cestode development proceeds.

The cellular constituents of the post-embryonic stages are variable. Transverse myofibers are present at the three day stage and become more pronounced by the fifth day when an orderly alignment of transverse and longitudinal peripheral fibers occurs. After three days in vivo a loose aggregation of cells is present about the periphery of the organism. Cytoplasmic processes extend randomly toward the interior. By five days in vivo the number and length of the cytoplasmic processes from individual cells increases with development. A greater variety of cell types are present by the fifth day.

This investigation was supported in part by the National Institutes of Health Fellowship 7 F02 AI40 214-01A1 from NIAID.

135

Rostellar morphogenesis in Taenia crassiceps P.M. MOUNT

The sequence of morphogenetic events of rostellar development in T. crassiceps cysticerci was studied by light and electron microscopy. The tegument of the rostellum undergoes a series of developmental changes which are believed to be related to events in hook formation. Rostellar hooks appear to originate through the enlargement of specialized tegumental microvilli. Prior to the initiation of hook morphogenesis the tegument is relatively undifferentiated, containing few mitochondria and other formed inclusions. As the hook forming microvilli enlarge, numerous mitochondria are evident in the tegument. At a later stage the hooks become surrounded by a modified tegument that is filled with electron dense vesicles apparently produced in the subtegumental cell bodies. Morphological evidence suggests that these vesicles contribute to the secondary thickening of the hook blade.
(Supported by grants from NIH (AI 08673, 5 TI GM 669) and NSF (GM 7276)).

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Origin and Possible Utilization of Small Dense Granules in Oocytes of Ascaris lumbricoides. W. EUGENE FOOR

Ultrastructural studies reveal that oocytes, but not oogonia, in the ovaries of Ascaris lumbricoides possess aggregations of dense material closely applied to the external surface of the apical plasma membrane. Tangential sections of the ovary, showing surface views of oocytes, reveal that the dense material is arranged in a net-like fashion and is comprised of 250-300 A particles. Where the dense material is most evident the apical oolemma of each oocyte is characterized by the presence of numerous infoldings. These membrane invaginations apparently progress until they separate from the plasma membrane. The dense material present in the resultant cytoplasmic vesicles is either incorporated into large 4 to 5 u inclusions or condensed to form .2 to .3 u granules. The small granules, which gradually increase in number during oocyte maturation, are not utilized for shell formation following fertilization. They are apparent in the cytoplasm throughout all stages of embryonic development and, although reduced in number, they are still present in the infective stage larvae where they are confined largely to the undifferentiated gut cells. It is proposed that the granules serve primarily as a structural protein.
(Supported by NIH Grants AI-04953, GM-08776, AI-04919 and 2T01-AI-00002.)

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Ultrastructure of the Rectal Gland Cells in the Larva of Dirofilaria immitis HARLEY G. SHEFFIELD* and PAUL P. WEINSTEIN

The rectal complex in developing larvae of Dirofilaria immitis consists of three cells, presumably G_{2-4} of the microfilaria, an anal vesicle with a pore opening to the exterior, and

the anal plug which closes the pore.

Electron microscope study indicates that the rectal cells are secretory in function. Each is elongate and has a large vesicular nucleus with a prominent nucleolus. The nucleus is surrounded by a double membrane nuclear envelope which is perforated by numerous pores. Adjacent to the nucleus are several Golgi complexes, each with typical flattened cisternae and vesicles. The remaining cytoplasm is filled with rough-surfaced endoplasmic reticulum free ribosomes and elongate mitochondria with lamellar cristae.

The three rectal gland cells surround the anal vesicle with their adjacent lateral margins being attached by typical tight junctions. The apical end of each cell borders the anal vesicle and has an elaborate meshwork formed by the highly infolded plasma membrane. Adjacent to this meshwork the lumen of the anal vesicle is filled with an amorphous material which protrudes through the anal pore forming the anal plug. Cuticle lines the pore and the anal vesicle up to the apical portions of the rectal gland cells.

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Ultrastructure of the Ovejector Diverticulum of Rhigonema infecta (Nematoda: Rhigonematidae).

George S. Hamada

The ovejector diverticulum is a large sac-like structure situated between the vagina vera and vagina uterina in female Rhigonema infecta. This structure consists of a cellular layer enclosing an internal acellular matrix. The cellular layer, studied with the electron microscope, was found to consist of two cell types, flat epithelial cells and elongate fusiform muscle fibers. The epithelial cells contain mitochondria, rough endoplasmic reticulum and luminal papillae lined with electron dense bodies. The muscle fibers contain mitochondria, rough endoplasmic reticulum associated with the plasma membrane and myofilaments, 50 and 150A in diameter, which undulate through the muscle fiber. The presence of muscle fibers in the ovejector diverticulum suggests that the structure is capable of contraction and may aid in egg expulsion. (Supported in part by USPHS fellowship 5-F1-GM-32,731-02 from NIGMS.)

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Ultrastructure and Cytochemistry of the Intranuclear Inclusion bodies of the Intestinal Cells of Obeliscoides cuniculi

M.A. FERNANDO

The inclusion bodies found within the intestinal cell nuclei of O. cuniculi were studied using electronmicroscopic and cytochemical techniques. In adult intestines each nucleus contained several inclusions. Among them three morphologically distinct types were recognized. The most commonly seen inclusion body was rounded, amorphous and about 2u in diameter at its largest. The other two, the one bar shaped and the other rhomboidal, were considerably smaller and crystalline in appearance. All three types were very osmophilic and appeared dense and structureless even at high magnification. The inclusions found within the nuclei of the early 5th stages, however, appeared fibrillar and were arranged in sheaflike bundles in close proximity to the nucleolus. These several types of intranuclear inclusion bodies were characterized histochemically and by enzyme digestion techniques.
(Supported by the National Research Council of Canada and the Ontario Department of Agriculture and Food).

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Influence of Fertilization on Ovogenesis in Ancylostoma caninum,

L. F. LeJAMBRE* and J. R. GEORGI

Fertilization is necessary in Ancylostoma caninum for the formation of the inner layers of the egg shell. These inner layers are formed after telophase I by coalescence of secretory granules that rise to the surface of the ovum from within. Eggs that lack these layers are very fragile and easily destroyed. The outer layers of shell are formed by small particles which appear near the ovarian end of the uterus. These particles coalesce on the surface of the eggs after completion of telophase I. The rate of egg laying appeared to be similar for fertilized and unfertilized females. Both unfertilized and fertilized worms laid eggs in vitro and their uteri contained similar numbers of eggs. Meiosis stopped at telophase I in unfertilized eggs and was followed by degeneration of the chromosomes. The diploid chromosome number in A. caninum is 2N = 12. (This work was supported by NIH GRS grant number FR05462 and by the State of New York.)

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Surface ultrastructure in developing Moniliformis dubius.

R. D. WRIGHT* and R. D. LUMSDEN

The acanthor tegument overlies a central cellular mass that exhibits regional differentiation. Surface pores are continuous with a system of canals located within the shallow hypodermis. Mitochondria and numerous thick-walled vesicles are present in the hypodermis and scattered throughout the central cytoplasm. The adult tegument contains elongate anastomosing canals extending from the periphery of the body wall into the hypodermis where they end blindly at a depth

of about 4 μ. There is spacial continuity of these tubules with the free surface, via pores defined by the tegumentary plasmalemma. This membrane also forms a continuous lining over the lumenal surface of the canals. The trilamenate plasmalemma measures about 120 A at the outermost margins of the body wall and 90 A at the hypodermal canals. The pore-canal complexes are interpreted as stable, structural specializations for the amplification of the free surface of the parasite available for chemical interchange with its environment. (Supported by grants from the NIH (A108673, 5T1GM669), NSF (GB7276) and Career Development Award (KO4-AI23449) (to RDL) from the USPHS).

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Nemertean Epidermis: A Host-Parasite Interface, JOHN A. OAKS*

The nemertean Carcinonemertes carcinophila maintains a symbiotic relationship with the blue crab Callinectes sapidis and several other species of crab. These rhyncocoels are commonly found on the egg masses or between the gill lamellae usually within secreted mucous sheaths. Material comprising the gill sheaths appear layered in the light microscope. At the level of electron microscopy the layering can be attributed to differing densities of fibers, irregular dense granuals and fragments of membrane bound cytoplasm. Similarly appearing material is sequestered in gland cells of the dermis. The free surface of the epidermal chief cells possess both cilia and microvilli. Fine filamentous projections adorn the outer lamella of microvillus plasma membrane. The cytoplasm proximal to the surface plasmalemma contains many membrane bound mitochondria and ciliary basal bodies with extremely long rootlet systems. The basal portions of the cells interdigitate with neighboring chief cells by means of folds.

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The Growth and Survival of Amastigotes of Leishmania donovani in Cultures of Peritoneal Macrophages, DONALD W. TWOHY*, JAMES R. FORD, and VIRGINIA FUNG

Cultures of peritoneal macrophages from normal mice supported the multiplication of amastigotes of Leishmania donovani for 2-5 days. The growth period was followed by an abrupt decline in the number of parasites, but the surviving population seemed to persist for the life of the culture. There was no evidence that the host cells developed an acquired resistance to the parasites in culture. Superinfection of macrophages produced a supplemental increase in parasite numbers comparable to that of controls receiving only the initial infection. Nearly all the amastigotes were engulfed by the macrophages within 6 hours after infection. Usually less than 5 percent of the surviving parasites were found in the medium in samples collected between 6 and 72 hours after infection. It seemed unlikely that they were killed by exposure to the extracellular environment since amastigotes survived as long as 72 hours in cell-free culture medium at 37C. Increasing the inoculum to over 100 amastigotes per macrophage gave no increase in the percent of extracellular parasites. Parasite destruction or multiplication in macrophage cultures seems entirely dependent on the nature of the intracellular association between the parasite and the host cell. (Supported in part by U. S. Army Medical Research and Development Command Contract DADA 17-67-C7142.)

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Cinemicrographic Observations on Refractile Body Changes in Sporozoites of Poultry Coccidia in Cell Culture, RONALD FAYER*

Changes in the shape and location of refractile bodies in living intracellular Eimeria tenella, E. adenoeides and E. meleagrimitis sporozoites in monolayer cultures of bovine embryonic kidney cells were studied and recorded by time-lapse cinemicrography (4 frames/min) using phase-contrast microscopy. For intervals up to 24 hr after sporozoite inoculation, Rose perfusion chambers and double coverslip preparations were examined on a microscope in an enclosure maintained at 39 C. In sporozoites with 2 refractile bodies, 1 anterior and 1 posterior to the nucleus, the anterior body moved posteriad to a position alongside or behind the nucleus and, within 15 min or less, merged with the posterior body. After this occurred, the sporozoite contained a single refractile body. Prior to, during, and after the merger of refractile bodies, finger-like projections appeared randomly along the anterior margin of the posterior body. These underwent lateral as well as up and down movements, became detached from the refractile body, and were observed in the sporozoite cytoplasm. Similar projections were observed along the periphery of the anterior refractile body in a small number of E. adenoeides and E. meleagrimitis sporozoites.

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Eimeria tenella: Asexual and Sexual Development in Cell Cultures, R. G. STROUT* and C. A. OUELLETTE

Asexual and sexual development of Eimeria tenella occurred following inoculation of primary cultures of embryonic chick kidney cells with motile sporozoites. The cultures, grown in Eagles Minimum Essential Medium supplemented with 10% newborn calf serum, were incubated at 40C to 41C. Devel-

opment of the parasite in vitro closely paralleled that of in vivo, for first generation schizonts matured 2 to 3 days after inoculation and second generation trophozoites and immature schizonts occurred after approximately 72 hours, becoming completely segmented with merozoites following 96 to 120 hours of incubation. Cell invasion and subsequent development of second generation merozoites resulted in immature gametocytes, distinguishable at approximately 144 hours after inoculation. The gametes matured 24 to 36 hours later at which time we also saw mature schizonts suggestive of a third generation.

In live, unstained preparations intracellular sporozoites and merozoites moved easily in either direction. Within the elastic walls of a mature schizont, all the merozoites suddenly became active although their subsequent release was usually gradual.
(Supported in part by NIH Grant AI 05745-05, and a grant-in-aid from Eli Lilly Co.

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Development of Eimeria alabamensis in Cell Cultures, J. ROBERT SAMPSON* and DATUS M. HAMMOND

Excysted sporozoites of Eimeria alabamensis, whose stages in the calf are intranuclear, were inoculated in doses of 250,000/ml into various monolayer cell cultures. Development occurred in secondary cultures of embryonic bovine trachea and synovial cells and in established cell lines of Madin-Darby bovine kidney (MDBK), Syrian hamster kidney, embryonic bovine trachea (EBTr) and human intestine. Coverslips were examined with phase-contrast microscopy at 6 hrs and then daily for 4-10 days. After examination, coverslips were fixed in Schaudinn's and stained with iron hematoxylin and PAS-AO. Sporozoites penetrated cells in all cultures and by 24 hrs after inoculation many were located close to the host cell nucleus. In material fixed 2 days after inoculation, 1-4% of the parasites were intranuclear. Some sporozoites transformed into trophozoites; in others the nuclear division occurred before the sporozoite had undergone a change in shape. Mature schizonts, which were most numerous in EBTr cells, usually appeared in these cells by 2 days and contained 4-10 short, stubby merozoites measuring 3.5-5.0 μ long by 1.5-2.2 μ wide. Mature schizonts in MDBK cells, usually not seen until 3 days after inoculation, contained 8-12 merozoites measuring 5.0-7.1 μ long by 1.2-2.0 μ wide. Merozoites, some of which were binucleate, had refractile body-like structures, which stained orange with PAS-AO. Merozoites usually left the host cells in pairs without undergoing any constriction, suggesting disintegration of the host cell membrane. Merozoites which had invaded new host cells and second generation trophozoites were observed 4 days after inoculation.
(Supported by NIH Grant AI-07488.)

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Development of Eimeria callospermophili in Cell Cultures, CLARENCE A. SPEER* and DATUS M. HAMMOND

Various primary cells, cell lines and established cell lines of bovine and ground squirrel origin were used to study in vitro development of Eimeria callospermophili from the Uinta ground squirrel, Spermophilus armatus. Monolayers in Leighton tubes were inoculated with sporozoites and examined with phase-contrast microscopy at 2-10 hr intervals for 48-72 hr. Monolayers were fixed in Schaudinn's or Zenker's fluid and variously stained. Sporozoites underwent development in all types of cells tested. At first, each intracellular sporozoite had an anterior and an unusually large posterior refractile body, whereas at 6-10 hr, most had only the posterior refractile body. At 3-15 hr, sporozoites became enlarged and nuclear divisions occurred, resulting in the formation of sporozoite-shaped schizonts, each with 2-12 nuclei. Usually, after 12 nuclei were present, these schizonts became spheroidal; several small refractile bodies were present. Merozoites were formed at the periphery of the schizont. Mature first-generation schizonts, with 6-14 merozoites, were first seen 15 hr after inoculation in embryonic ground squirrel primary cells. Merozoites had spherical anterior and posterior bodies similar in appearance and staining characteristics to the refractile bodies of the sporozoite. They were eosinophilic with H and E, orange with PAS-AO, and slightly basophilic with iron-hematoxylin. A few multinucleate second-generation schizonts, some of which were in the early stages of merozoite formation, were seen 44-48 hr after inoculation. (Supported in part by NIH Grant AI-07488.)

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Temperature Tolerance of Echinostoma revolutum on the Chick Chorioallantois, B. FRIED* and D. A. FOLEY

To determine temperature tolerance of Echinostoma revolutum on the chick chorioallantois, 7-day-old preovigerous flukes were transferred to membranes for 7 days at 30, 32.5, 35, 37.5, 39 and 41 + 1 C. Live flukes were recovered from the upper and lower surface of the chorioallantois, in the albumen, and on the surface of the embryo in eggs incubated at 30 to 39 C. At these temperatures chorioallantoic-worms fed on blood. Histochemical tests for egg shell precursor material were positive in 32.5 to 39 C chorioallantois-worms; 35 to 39 C chorioallantoic-flukes were ovigerous, whereas those from the albumen were not. Eggs teased from 37.5 and 39 C flukes and incubated in tap water developed or hatched. Ovigerous worms varied considerably in size, some of which were not significantly larger than preovigerous or non-ovigerous flukes.
(Supported by NIH Grant AI-06835.)

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Effects of some vitamin antimetabolites on in vitro development of Hymenolepis diminuta,
LARRY S. ROBERTS* and FAITH N. MONG.

Hymenolepis diminuta was cultured in vitro by a method modified from that of Schiller (1965, J. Parasit. 51:516). The worms were recovered from rats at 6 days postinfection and cultured for 12 days. Development was completely inhibited by 400 μM desoxypyridoxine (DOP) in the medium, and the inhibition was reversed by the presence of an equimolar amount of pyridoxine in addition to the antimetabolite during the culture period. A concentration of 40 μM DOP had no effect on development, while 200 and 300 μM DOP caused partial inhibition. Development was completely inhibited also by 17 mM and partially by 0.17 mM 6-aminonicotinamide. The presence of an equimolar concentration of nicotinamide was not sufficient for complete reversal of the effects of the 6-aminonicotinamide. Interestingly, 17 mM nicotinamide alone in the control medium severely interfered with reproduction in the worms, although growth itself was not inhibited. The presence of 50 uM 4-amino pteroyl glutamic acid (aminopterin) or 2.23 mM oxythiamine did not affect growth or reproduction of H. diminuta under the conditions of the experiments. (Supported in part by NIH grants AI-06153 and 5 TOI-AI-226.)

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Development in vitro of Hymenolepis microstoma from cysticercoid to adult,
JAMES S. SEIDEL*

Excysted cysticercoids were cultured in monophasic and diphasic media, the latter modified from Schiller (1965 J. Parasit. 51: 516) and Voge and Seidel (1968 J. Parasit. 54: 269). Liquid media were: GIBCO NCTC 135 and Triple Eagle's with and without horse serum, or Hank's balanced salt solution. Varying amounts of hemin, protoporphyrin IX, sodium taurocholate, and glucose, were incorporated into the medium in different experiments. Effects of changes in the physical environment were examined by altering gas composition, pH, surface of the agar and by agitating culture vessels. In monophasic media limited growth without segmentation occurred. When hemin was added to these media, strobilization was initiated on the 6th day of culture; at 4 weeks maximum length was 2.1 mm, with 60-70 segments and primordia of gonads. Organisms on nutrient agar with hemin added to the overlay attained a maximum length of 32 mm and contained semi-gravid segments after 3 weeks. In a diphasic system with a gas phase of 90%N 5%CO_2 5%O_2, growth and differentiation during the first 10 days was comparable to that reported in vivo; thereafter strobilization rate and egg production were retarded. Semigravid segments were observed on the 13th day, however the eggs were immature and lacked an outer shell. Average length after 21 days in culture was 65 mm. Longer organisms were grown in a closed diphasic system with air as a gas phase. In all systems most mature segments and organs were morphologically normal. (Supported by USPHS Grant T1-AI-132-08 and USPHS 5 R01-AI-07332.)

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Environmental Effects on Strongyloides fülleborni in Culture,
E. J. BUECHER*, E. HANSEN, and A. K. BERNTZEN

In fecal culture, development of the rhabditoid cycle was affected by anaerobiosis and crowding. Cultures were initiated with known numbers of eggs deposited on worm free feces spread on non-nutrient agar slants. In some cases the eggs were covered with feces. Filariform larvae and males developed in thinly spread feces incubated in air. When incubated under thick feces, or in gas phase with decreased oxygen and increased carbon dioxide, rhabditoid females developed also. These females deposited eggs from which a second generation of females developed. In heavily parasitized feces only males and filariform larvae developed. Monoxenic cultures with E. coli could not be established.

Axenic cultures were initiated with eggs treated with antibiotics. The chemically defined medium CbMM was diluted, modified, and bicarbonate added. Supplements of CEE, serum,or crude growth factor from liver or yeast were added. Microaerophilic conditions were established by additions of the reducing agents, cysteine and glutathione, and by change in gas phase to low oxygen and increased carbon dioxide. Rhabditoid males and females and filariform larvae developed. The ova of the females were few and showed abnormal segmentation.

Axenic cultures of the filariform stage were initiated with larvae that had penetrated through excised mouse skin. Culture conditions were medium 115 with supplements, pH 7.0-7.2, 37 C, and gas phase 10%O_2-10%CO_2-80%N_2. Change of gas phase at intervals to 40%CO_2-60%N_2 induced molting. Growth and gonad development were observed.
(Supported by NIH grants AI-7359 and AI-07218.)

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In vitro Cultivation of Oesophagostomum columbianum in a Simplified Medium with Subsequent Isolation of Parasite Antigens,
J. T. McL. NEILSON*

O. columbianum can be cultured in vitro from the infective third to fourth larval stage in a 'complex' medium which includes chick embryo extract (CEE) and sheep serum. The complexity of this culture medium complicates subsequent isolation of excretions and secretions produced by the parasite during in vitro development. To overcome this difficulty, CEE and sheep serum were separated, by gel filtration on Bio-Gel P6, into two fractions and the non-excluded fraction which contains components with a molecular weight less than 6000, was used in the preparation of the 'simplified' culture medium. The rate and degree of development of O. columbianum in this medium was similar to that obtained with the 'complex' medium. 50% of the larvae reach the fourth stage after 9 days and begin to die after 17 to 20 days in culture. No significant development beyond the fourth stage was obtained. Simplified medium in which the parasite had developed was subsequently applied to a Bio-Gel P6 column. As the simplified medium contained no components with a molecular weight greater than 6000, the excluded fraction consisted of

excretions and secretions along with somatic material from dead worms, relatively free of medium components.

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Feasibility of Utilizing In Vitro Grown Parasitic Nematodes to Detect Anthelmintic Activity. S. E. LELAND, JR.

The exact manner by which anthelmintics exert their activity against parasites is in general difficult to determine. Part of the difficulty lies in the fact that such studies must be conducted in a situation complicated by the presence of both host and parasite. We have used the in vitro grown parasitic stages of Cooperia punctata to detect direct anthelmintic activity of Trichlorfon, Coumaphos, and Naphthalophos in contrast to activity possibly due to metabolic or host-altered products of the compounds. Medium Ae (Leland, J. Parasit. 49: 600-611, 1963) was used. Experimental design included variation in the concentration of the organic phosphate and age of the nematode when the compound was introduced. Results indicate direct anthelmintic activity at a concentration (ppm in medium Ae) greater than 1 to 10 for Trichlorfon, 0.1 to 1.0 for Coumaphos, and 10 to 100 for Naphthalophos in 13, 22, 29, and 43 day old cultures. Compounds were active against the 3rd, 4th, and 5th stage adult nematodes. (Supported in part by NSF grant GB-7532 and a grant from the Chemagro Corporation).

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In Vitro Cultivation of Ancylostoma tubaeforme from Egg to Fourth Stage Larvae and Maintenance of Adults. G. F. SLONKA* and S. E. LELAND, JR.

Ancylostoma tubaeforme was cultivated in vitro from the egg to advanced fourth-stage in a modification of Ae medium (Leland, J. Parasit. 49: 600-611, 1963). Ae was modified by including cat serum in the medium in place of calf serum. Advancement from egg to third stage was completed in eleven days and from third to fourth stage in 31 days in the same system. When third stage larvae originating from vermiculite-fecal cultures were inoculated into the medium, advancement to fourth-stage was made in 7 days. However, the number of larvae reaching fourth-stage did not exceed 1%. Many of the fourth-stage larvae died in the process of ecdysis to fifth stage. When the serum component of the medium was from immature rather than mature cats, advancement time from third- to fourth-stage was reduced by half. Adult worms from in vivo were also maintained in the modified Ae medium 49 days with the females laying eggs for 41 days. Copulation was observed in vitro. (Supported in part by NSF grant GB-7532).

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In Vitro Cultivation of Cooperia punctata from Third-stage Larvae to Adults which Produced Eggs that Hatched and Developed to Second-stage Larvae. G. L. ZIMMERMAN* and S. E. LELAND, JR.

Third-stage Cooperia punctata larvae were processed and inoculated into a recent version of Ae medium (Leland, J. Parasit. 49: 600-611, 1963) and incubated at 38.5 C in a roller drum. The nematodes were transferred into fresh culture medium every 7 days. Eggs were first detected in culture after 34 days and on day 45 cleavage had taken place with many eggs in the 32 cell stage. Embryonated eggs containing active larvae were first detected on day 48. Daily observation thereafter revealed increasing numbers of first-stage larvae in the medium. Small numbers of second-stage larvae were also detected. By day 57 embryonated eggs were present in 7 of 11 cultures. Of these 7 cultures 4 contained many first- and second-stage larvae. (Supported in part by NSF grant GB-7532).

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The FDA Looks at Veterinary Anthelmintics, JOHN B. POOLE* and JAMES H. MARK

The Bureau of Veterinary Medicine (BVM) has been criticized, from time to time, in regards to its reviewing of new drug applications for veterinary anthelmintics. It would appear that there has been some misunderstanding on the part of both this Federal Agency and industry. Because it is considered that each new drug should be judged on its own merits, it is difficult to lay down hard and fast guidelines. It is the responsibility of BVM to make certain that all drugs and devices intended for the treatment of animals are safe and effective. Proper labeling must accompany such articles; it must be informative as to recommendations for use and also bear precautions against misuse. If the drug is intended for food-producing animals, the labeling must bear instructions regarding its use which will preclude its becoming a residue in edible products (milk, meat, or eggs) derived from treated animals. The following are some of the subjects evaluated in an NDA: Safety and toxicity studies including fertility, abortive and teratological information; EPG; critical and controlled trials; foreign country field trials and their considered value; laboratory versus field studies and data submitted by outside investigators, labels and labeling listing the species of animals and the claims and directions for use of the product. Some examples are given of acceptable and non-acceptable data that have been submitted. Cooperative assistance that is being offered by the Bureau of Veterinary Medicine for the mutual benefit of industry and the Federal Government is mentioned.

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Effects of Two New Triaminoquinazolines on Malaria in Animals, PAUL E. THOMPSON*, ANITA BAYLES and BRONISLAWA OLSZEWSKI

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Research to develop new agents that may be useful against drug-resistant malaria has led to compounds PAM 1392 [2,4-diamino-6-(3,4-dichlorobenzylamino) quinazoline] and CI-679 [2,4-diamino-6(3,4-dichlorobenzyl)nitrosaminoquinazoline]. Sensitive and drug-resistant lines of Plasmodium berghei in mice and sensitive lines of P. cynomolgi or P. knowlesi in rhesus monkeys were used to assess antimalarial activity. All infections were induced with blood forms of the parasites. In the treatment of mice orally, PAM 1392 was several-fold more potent than quinine, acted rapidly, was synergistic with sulfadiazine, and was effective against lines of P. berghei that are highly resistant to chloroquine, DDS, cycloguanil HCl, or pyrimethamine. PAM 1392 had no appreciable repository activity in mice. PAM 1392 acted somewhat more slowly in monkeys but when given appropriately cured infections of P. cynomolgi or of P. knowlesi. PAM 1392 inhibited nuclear division of the parasites. CI-679 orally in mice was approximately 500-fold more potent than quinine, acted rapidly, was synergistic with DDS and was effective against the above drug-resistant lines of P. berghei. Against P. cynomolgi in monkeys, CI-679 orally was highly potent and curative and parenterally prevented patent infections for several months. CI-679 also had repository activity for several weeks in mice. Both compounds appear to represent a new mode of action among known antimalarials.

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Effect of Catalpa bignonioides on the Parasites of Poultry, PAUL D. HARWOOD*

Since wood of C. bignonioides extracted with isopropyl alcohol and used as a feed additive affects favorably the performance of broiler chicks, tests with parasitic infections were conducted. Experimental chickens were stratified by weight and the extract administered in the feed. Extract at 0.0011, 0.017, and 0.033 per cent had no effect upon the establishment or development of the cestode, Raillietina cesticillus. At the same levels extract did not reduce the death rate from experimental infections of Eimeria tenella, however, at the median level weight gains were improved very slightly during the infection. Twenty controls gained an average of 118 grams, and 20 similarly infected principals receiving 0.017 per cent Catalpa gained 126 grams. Difference of 8 grams per bird approached significance at the 5 per cent level, and has proved reproducible with or without other feed additives (nitrofurans). I conclude that extract of C. bignonioides wood has no worthwhile effect on infections with either of these two diverse parasites.

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New Agent for Treatment of Fascioliasis, W. C. CAMPBELL*, D. A. OSTLIND, R. F. RIEK and J. J. YAKSTIS

Studies in naturally and experimentally infected ruminants have shown that 3,5-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanilide is a highly potent agent for the treatment of Fasciola hepatica infections (Mrozik, et al., in press). Studies in sheep have shown it to be more than 90% effective in eradicating 6-week-old flukes at dosages of 10-15 mg/kg, and more than 90% effective against adult fluke at a dosage of 5 mg/kg. The drug is tolerated at a dosage of 60 mg/kg even in sheep with severe acute fascioliasis (inoculated with 1000 metacercariae each, 8 weeks before treatment). It is also effective, and well tolerated, when used orally or parenterally for the treatment of fascioliasis in cattle.

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Efficacy of Parbendazole Against Gastrointestinal Nematodes in Angora Goats, V. J. THEODORIDES*, G. C. SCOTT and MARVIN LADERMAN

Sixty Angora goats naturally infected with gastrointestinal nematodes were used in a controlled experiment. A single oral dose of 10 mg/kg parbendazole eliminated 99 to 100% of Trichostrongylus and Oesophagostomum; 94 to 100% of Ostertagia and Strongyloides were eliminated at 20 mg/kg. Nematodirus and Haemonchus were reduced by 99% at 30 and 60 mg/kg, respectively.

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Anthelmintic Efficacy of Maretin in Two Controlled Tests with Calves. D. D. COX* and M. T. MULLEE

The anthelmintic activity of 50 mg./kg. Maretin (naphthalophos) administered orally against mixed gastrointestinal nematode infections was determined in 2 controlled tests with 12 calves each. Calves in one test had naturally acquired parasite infections, while those in the second test were given approximately 72,800 third-stage larvae orally in 2 doses. In each test 4 calves were drenched, 4 received boluses and 4 were untreated controls. The effect of Maretin on the number of gastrointestinal nematode eggs in the feces of the treated cattle was similar in both tests. Reductions in number of eggs by 4 to 5 days after treatment were 96 to 100% by the drench and bolus treatments in both tests. Using parasites recovered at necropsy as a criterion, Maretin was 96 to 99% effective against all gastrointestinal nematodes and was very efficacious against Haemonchus, Ostertagia, Trichostrongylus and Cooperia spp. in both tests. Efficacy against Oesophagostomum was poor to moderate in the 1 test in which sufficient worms were present for evaluation. Three species of nematodes (T. longispicularis, C. mcmasteri and C. spatulata) not usually found in cattle throughout the United States were recovered. No signs of organic phosphate toxicity were observed after treatment.

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Efficacy of Baymix Against Gastrointestinal Nematodes in Two Controlled Tests with Calves. M. T. MULLEE* and D. D. COX

The anthelmintic activity of 2 mg./kg. Baymix (coumaphos) mixed in feed or topdressed on feed for 6 consecutive days against Haemonchus, Ostertagia, Trichostrongylus and Cooperia spp. was determined in 2 controlled anthelmintic tests with 12 calves each. In one test the calves had naturally acquired nematode infections and in the other the calves were inoculated orally twice and once intrarumenally with a total of approximately 102,658 to 121,669 third-stage larvae. In each test 4 calves received Baymix mixed in feed, 4 received Baymix in crumbles topdressed on feed, and 4 were untreated controls. The number of strongylina-type eggs in the feces of calves in both tests was reduced 89 to 99% after treatment with Baymix mixed in or topdressed on feed for 6 days. On the basis of nematodes recovered at necropsy, Baymix removed 90-99% of all gastrointestinal nematodes. It was very efficacious against the 4 predominant genera (Haemonchus, Ostertagia, Trichostrongylus and Cooperia) in 1 test and against 3 genera in the second test. Only moderate control of the fourth genus, Ostertagia, was obtained in the second test. No signs of organic phosphate toxicity were observed in either test. Baymix was palatable to calves when given in feed and topdressed on feed.

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Efficacy of Thiabendazole Against Two Ovine Isolates of Haemonchus contortus, M. L. COLGLAZIER*, K. C. KATES, and F. D. ENZIE

In three controlled anthelmintic trials, half of a total of 64 helminth-free lambs were each inoculated with 5,000 infective larvae of our Beltsville Parasitological Laboratory (BPL-2) isolate of H. contortus and half with 5,000 larvae of our Animal Husbandry Research Division (AH-2) isolate. In each of the three trials, half of the lambs infected with each isolate were treated with 50 mg/kg of thiabendazole 3 weeks postinfection, and the other half of the lambs served as untreated controls. In two trials the four experimental groups consisted of 4 lambs each, and in one trial 8 lambs each. All lambs were necropsied 4 weeks postinfection, and drug efficacy was calculated from total worm counts of medicated and control lambs. Following are the calculated drug efficacies for the two isolates of H. contortus in the three trials: Trial 1 BPL-2 78%, AH-2 65%; Trial 2 BPL-2 87%, AH-2 66%; Trial 3 BPL-2 62%, AH-2 27%. Analysis of variance of the data showed that the AH-2 isolate of H. contortus was significantly less responsive to thiabendazole at 50 mg/kg than the BPL-2 isolate. However, the calculated efficacies against both isolates were less than that commonly reported against H. contortus at the 50 mg/kg dose rate.

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Efficacy of Thiabendazole, l-Tetramisole, and Parbendazole Against Natural Infections of Helminths of Sheep, K. C. KATES*, M. L. COLGLAZIER, F. D. ENZIE, I. L. LINDAHL, and G. SAMUELSON JR.

Forty-five ram lambs were grazed on a helminth-contaminated pasture at Beltsville, Md. When the lambs had acquired substantial infections with various helminths, they were removed from pasture to a helminthologically sterile pen where they were separated at random into 5 groups of 9 lambs each. One group of lambs served as unmedicated controls, and 4 groups were treated with anthelmintics as follows: (1) thiabendazole at 50 mg/kg; (2) thiabendazole at 100 mg/kg; (3) l-tetramisole at 8 mg/kg; (4) parbendazole at 15 mg/kg. All lambs were necropsied 4-6 days posttreatment, and residual worm counts were made by standard techniques. Both mature and immature parasites of several species were recovered from the control lambs; Haemonchus contortus was the most numerous. For groups 1, 2, 3, and 4, respectively, the calculated efficacies of the treatments for all stages of parasites were as follows: H. contortus 48, 88, 99, and 79%; Ostertagia spp. 94, 99, 77, and 99%; Trichostrongylus spp. 99, 100, 99, and 100%; Nematodirus spathiger 90, 100, 96, and 83%; Oesophagostomum venulosum (5th-stage only) 98, 100, 100, and 99%. Fourth-stage nematodes were not removed as effectively as 5th-stage, except that l-tetramisole was effective against 4th-stage H. contortus. In all 5 groups of lambs the numbers of scolices of Moniezia expansa recovered were comparable, but the three anthelmintics, particularly parbendazole, appeared to reduce strobilar volume.

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New Agent for Treatment of Haemonchosis, J. R. EGERTON* and W. C. CAMPBELL

A new anthelmintic, 3,5-diiodo-3'-chloro-4'-(p-chlorophenoxy)-salicylanilide, has been found highly effective in the treatment of Haemonchus contortus infections in sheep. This compound was given as a drench, at 5 mg/kg and 10 mg/kg, to 2 groups of sheep that had been inoculated with Haemonchus larvae 8 days previously, and, at 10 mg/kg, to another group of sheep inoculated 22 days previously. Four similar groups were either untreated or treated with the new anthelmintic in combination with thiabendazole. At necropsy of the sheep treated 8 days after infection, the dosages of 5 and 10 mg/kg were found to have reduced the worm burden by 93% and 99%, respectively (in comparison to the untreated sheep). In the sheep treated 22 days post-inoculation, the dosage of 10 mg/kg reduced the worm burden by 99%. In the remaining groups, the new drug was found to be fully compatible with thiabendazole.

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The Effect of Levo-Tetramisole on Experimentally Induced Infections of Trichostrongylus axei and Ostertagia ostertagi in Calves, G.H. ROHRBACHER*, J. EMRO and E. WALETZKY

High efficacy with drenches of 8 mg. l-tetramisole (TMS)/kg. had been found in naturally acquired T. axei infection (av. of 96% removal). However, in work reported by Rubin and Hibler (1968) [Am. J. Vet. Res. 29(3): 545-548], there was relatively low efficacy (60% removal with a drench of 8 mg. per kg.) against 25-day-old T. axei in experimentally induced infections of cattle though efficacy was high against O. ostertagi and Cooperia spp. Three controlled tests were, therefore, conducted.

The sources of the nematodes used were identical to those of Rubin and Hibler (T. axei from Dr. H. Ciordia and O. ostertagi from Dr. H. Herlich), as was the route of inoculation, and the age of infection at time of treatment. Cooperia spp. were not included in these trials. In two of three experiments additional larvae were inoculated seven days prior to treatment. In addition to drenches of l-TMS used in all trials, intraperitoneal injection was tested in the third.

Drenches of 8 mg. l-TMS/kg. removed an average of 84, 86 and 96% of T. axei adults; 89 and 92% of T. axei immatures; 91, 96 and 97% of O. ostertagi adults; and 95 and 52% of O. ostertagi larvae. Intraperitoneally, 8 mg./kg. removed 94% T. axei adults and 82% immatures, and 98% O. ostertagi adults and 17% immatures.

These experimental infections were highly susceptible to 8 mg./kg. of l-TMS, as in past work with natural infections. The presence or absence of seven-day-old immatures at time of treatment did not alter efficacy on adults.

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Ultrastructural Changes Occurring in Dirofilaria immitis after Treatment with Caparsolate Sodium, CHIN-CHIU LEE

The body-wall, reproductive system, and the digestive tube of female D. immitis from an untreated dog and two dogs treated with Caparsolate Sodium were studied by electron microscopy. Little changes were observed in the cuticle, the muscle, and the reproductive system including microfilaria. Changes in the intestine were observed 24 hours following vermicidal treatment. Pigment granules, which are numerous in the anterior gut of normal worms, are reduced in number and size. They also seem to aggregate, lose their myelin figures, and have varying densities. Microvilli show disorganization and altered morphology. Organelles within the cells, namely mitochondria, Golgi complexes, and nucleus, appear normal. Dense deposits were found in the intestinal lumen, on the apical plasma membrane and microvilli, but absent within the cells. These deposits are assumed to be a protein-arsenical complex which alter the morphology and absorptive function of the striated border. This is considered the probable mechanism of the filaricidal action of the therapeutic agent.

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Movement of Long Chain Fatty Acids Across the Mid-Gut of Ascaris lumbricoides suum, CALVIN G. BEAMES, JR.* and GARY A. KING

The large daily output of eggs by Ascaris and the fact that the eggs have a very high lipid content indicate a high rate of lipid assimilation. Studies of the synthesis of long chain fatty acids by various tissues of the worm suggest that these compounds are formed de novo at very low rates. Apparently, most of the fatty acids incorporated into the eggs of the worm are obtained from the host. Experimental evidence suggests that Ascaris absorbs its nutrients through the intestine rather than the cuticle. The intestine of Ascaris can be removed easily and used as a "sac" preparation for in vitro determinations relating to the movement of nutrients. This technique has been employed in a series of experiments designed to determine the effect of various gases (95% N_2-5% CO_2, 95% O_2-5% CO_2, 95% Air-5% CO_2, and 99+% N_2) and the presence of glucose and bile salts upon the movement of palmitic, oleic, linoleic and stearic acid. Palmitic acid bound to albumin moved from the luminal to the coelomic solution at a rate of 5.6 mμ moles/sq.cm./hr. when glucose was present and 95% N_2-5% CO_2 was the gas phase. Movement was significantly reduced when glucose was omitted. Movement was reduced when gases other than 95% N_2-5% CO_2 were employed in the system. Bile salts in the luminal solution facilitated the movement of the acid across the mid-gut. Similar measurements were obtained with other long chain fatty acids. The results suggest that movement of long chain fatty acids across the mid-gut of Ascaris requires an energy source and CO_2. The observations agree with what is known of the worm's metabolism.

(Supported in part by USPHS, NIH Grant AI 06047)

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Localization and Distribution of DNA in Nuclei of Developing Ascaris lumbricoides var. suum, S. R. SYLK*

Studies of the nuclear distribution pattern in developing stages of Ascaris lumbricoides var. suum have been undertaken using a simplified method for the localization of DNA. The technique for demonstration of this nucleic acid within the nuclei involves the use of a recently developed fluorochrome. This compound, a bis-benzimidazol derivative has been shown to possess a high degree of specificity for DNA. These studies have demonstrated that following a 10 minute staining period in a 1:100,000 dilution of the fluorochrome, the nuclei of embryonating eggs, developing larvae, adult worm cross sections and animal tissues exhibit a brillient blue fluorescence when examined with ultra-violet light. Under these conditions, the developmental changes which take place in this nematode as it progresses from a fertilized single cell egg to a sexually mature adult worm have been studied. Additionally, this system is applicable to developmental studies on a wide variety of helminth and protozoan parasites. As yet, its role in studies of DNA metabolism and synthesis has not been established, however, such investigations are in progress. (Supported by USPHS Grants AI 00302 and AI 06202).

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Mouse Peritoneal Monocyte Reactions to Ascaris suum Larvae
E. L. Jeska*

Pyroninophyllic peritoneal monocytes adhere closely to surfaces of infective Ascaris suum larvae inoculated into peritoneal cavities of previously infected mice. During the course of an infection produced by stomach intubation of embryonated eggs in these mice, larvae migrated to peritoneal cavities on days 4, 5 and 6 after dosage. Peritoneal monocytes adhered to these larvae, underwent transformation, mitosis, and stained with pyronin.

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Lysozyme Action in the Symbiotic and Symbiont-free Cockroach, D. R. A. WHARTON*

Cockroaches harbor intracellular bacterial symbionts which are transmitted through the egg. Removal of the symbiont by heat, antibiotics or egg-white lysozyme (EWL) is followed by a reduction in reproductive capacity, viability and growth rate which has been attributed to loss of the symbiont. This view has been furthered by the claim that EWL, which induces a rapid removal of the symbiont, is non-toxic. EWL injected into the symbiont-bearing American cockroach not only destroyed the symbiont, but increased blood volume and tissue fluid, increased coagulation time, increased most of the amino acids, the Na^+/K^+ ratio in the blood, and caused tissue injury. Injection of a second dose of EWL into adults previously made aposymbiotic by a first injection caused an increase in blood volume and coagulation time. F_1 adults from cockroaches sterilized of their bacterial symbiont by means of antibiotics have a greatly increased content of most amino acids, but less tyrosine. Injection of EWL into these F_1 adults increased blood volume and coagulation time, increased some amino acids, and increased the Na^+/K^+ ratio of the blood. The evidence indicates that EWL is directly toxic to the host tissue and its toxicity is independent of products of bacterial disintegration. It may be sufficient to explain the reduction in reproductive capacity and other deficiencies that follow elimination of the symbiont. A role for lysozyme in inflammatory processes is suggested.

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A Common Mechanism of Immunity for Intracellular Infections, ALICE YURCHISION* and JACK S. REMINGTON

Recently we reported that mice infected with the obligate intracellular parasite Toxoplasma gondii were resistant to the facultative intracellular bacteria Listeria monocytogenes and Salmonella typhimurium in doses lethal to normal mice. The present studies were undertaken to determine if these observations are reproducible with other phylogenetically unrelated intracellular organisms and to define possible mechanisms underlying this "heterologous" resistance phenomenon. Mice were chronically infected with another intracellular protozoan, Besnoitia jellisoni, and challenged with a number of unrelated intracellular pathogens. These experiments revealed that Besnoitia-infected mice were protected against challenge with the intracellular bacteria Listeria monocytogenes, Salmonella typhimurium and Brucella melitensis, as well as against challenge with the intracellular protozoa Toxoplasma gondii. Besnoitia infection in mice also conferred complete protection against challenge with ordinarily lethal doses of Mengo virus. Peritoneal macrophages from normal mice and from "immune" mice infected with Besnoitia, Toxoplasma or Listeria were challenged in vitro with one of the organisms used in the in vivo experiments (e.g., Listeria or Toxoplasma). Definite resistance to necrotization by either Listeria or Toxoplasma was always clearly evident in monolayers derived from the infected mice. In summary, these findings support our initial hypothesis that common mechanisms of immunity exist for intracellular infections per se, and the in vitro studies indicate that the macrophage system is the effector arm of the observed resistance.

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Immunoelectrophoretic comparisons of some Entamoeba antigens.
M. N. Lunde* and L. S. Diamond

Antisera were developed in rabbits against antigen prepared from different strains of Entamoeba. Immunoelectrophoresis of these antigens against homologous antisera showed that E. histolytica strains 200:NIH and HK-9, were similar and characteristically different from strains 301:NIH and F-22, which in themselves were similar. Antigens from E. histolytica-like Laredo type amebae (strains Laredo and Huff) and the reptilian ameba, E. invadens (PZ) were similarily examined and characteristic patterns observed.

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Protective Effect of Noninfective Forms of Trypanosoma cruzi in Culture-initiated Infections, ROBERT G. YAEGER* and ELSA L. WINSOR

An explanation was sought for the lack of significant infections when cultures of a virulent strain of Trypanosoma cruzi are inoculated into susceptible animals. Four groups of mice were inoculated as follows: I, 10^8 live culture forms; II, 10^8 live culture forms and 50 blood trypanosomes; III, 10^8 sonicated culture forms and 50 blood trypanosomes; IV, 50 blood trypanosomes. Metacyclic trypanosomes were fewer than 1 in 5×10^4 culture

forms. Culture forms were given subcutaneously and blood forms were given intraperitoneally in the 1st experiment; routes were reversed in the 2nd experiment; all inocula were given subcutaneously in the 3rd experiment. A 4th experiment was done in the same manner as the first except that 5000 blood forms were used for groups II, III, and IV. Results: (1) live culture alone produced only light infections with no deaths; (2) 50 blood forms alone resulted in uniformly fatal infections; (3) simultaneous inoculation of culture forms with 50 blood forms decreased mortality by 90% in the 1st experiment, by 100% in the 2nd experiment, and by 40% in the 3rd experiment; (4) simultaneous inoculation of sonicated culture forms with 50 blood forms had no protective effect; (5) in the first 3 experiments survival time of group II mice which died was significantly longer than that of groups III and IV; (6) when the number of blood forms was increased to 5000, all mice in groups II, III and IV died within 18 days. These observations indicate the importance of the ratio of infective: noninfective forms in studies on the virulence of T. cruzi strains in culture, and suggest that the living noninfective stages exert an immunogenic effect on the host. (Supported in part by NIAID Grants AI-00977 and AI-00002.)

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Action of the thymus extract in experimental Chagas disease. *

J. Ottilio Machado

Observations on thymus extract inoculation in rats with chronic Tripanosomosis amerinana showed an acute reactivation of the process and an increase of parasitemis.

Two types of thymus estract were tested: saline total extract(ph-6,8) and thymus lipoide(ph-5). The latter showed a greater inhibitory activity on the defense mechanism.

Control was maintained by the circulating Trypanosoms countings and hispathology of the cardiac muscle.

Enhanced aggressivity was observed in the peripheric tissue with acute bleeding foci, and many Leishmannioid formas.

In view of these results, we believe that the thymus extract can act on the defense mechanism by reducing the rate of protective antibodies in Chagas disease.

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Immunologic Selection for a Mutant or Relapse Strain of Plasmodium lophurae in White Pekin Ducklings, R. M. CORWIN* and H. W. COX

Serum antigens (SA) from each of three hemosporidian infections proved immunogenic in ducks challenged with Plasmodium lophurae. Half of the immunized ducks invariably survived challenge while those that succumbed showed a rise in parasitemia similar to that seen in non-immunized (control) birds. Challenge of immunized ducks with Plasmodium "spartani", another highly lethal duck parasite, resulted in 100% survival in the immune group.

Mortality from P. lophurae challenge, therefore, seemed not attributable to inadequate immune response. Instead, fulminating infections in immune ducks might represent a relapse, or immunity-resistant (IR), "mutant" strain, and nonfulminating infections an immunity-susceptible (IS) population. Both IR and IS were isolated from immunized ducks and were subsequently passed in immunized birds. The IR strain killed both immunized and control birds with comparable effectiveness, whereas, the IS population failed to produce a fulminating infection in immune ducks but was as effective as the parent population in killing controls. We, therefore, feel that the IR strain is a "mutant" population which does not produce antigen and therefore is not influenced by antibody to nonspecific SA and that the IS population is homogeneous and stable to this type immunity.

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Immunization of Mice with Parasite Antigens of Plasmodium berghei and Babesia rodhaini

KYLE H. SIBINOVIC, Ph.D.

Immunogenic effects of Plasmodium berghei (KBG and Babesia rodhaini parasite antigens were determined in mice using parasites freed of host substances by differential centrifugation techniques. Forty-two per cent of the mice died of typical infections when one injection of the B. rodhaini parasite (BRP) antigen was used to immunize, and they were challenged with the homologous strain. Forty-eight per cent of the mice died when immunized with one injection of P. berghei (PBP) parasite antigens and challenged with the homologous strain.

All mice immunized with 3 injections of BRP antigen and 95% of the mice immunized with 3 injections of PBP antigen survived challenge with homologous strains.

All mice challenged with a heterologous species and all unimmunized control mice died with typical infections.

Serologic tests indicated that a serum antibody titer of 1:1280 or greater was necessary to survive a challenging injection. All surviving mice had antibody titers at 30 days past challenge that were greater than those obtained on day of challenge, as a result of immunization.

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Preliminary Studies on the Use of Ferritin-Conjugated Antibodies to Plasmodium berghei, Virginia A. A. Killby* and Paul H. Silverman

Pilot experiments to explore location of malarial antigens at electron microscope level involved asexual erythrocytic stages of P. berghei and anti-P. berghei antibodies induced in rats by repeated infection and in rabbits by immunization with French press- or saponin-prepared antigens. Anti-P. berghei antibodies were characterized by various serologic techniques including Ouchterlony analysis, complement fixation and immunoelectrophoresis before and after conjugation with ferritin by methods of Singer (1964) or of Falk (1968). P. berghei-infected cells or pellets of freed P. berghei were incubated with ferritin-labeled antibody preparations using indirect or direct approach. Several pretreatments and incubation schedules were carried out in effort to enhance intracellular localization of ferritin tag. Subsequent ultrastructural examinations revealed presence of ferritin in thin sections, but results were diffiqult to interpret. Absence or reduced amounts of ferritin associated with membrane material, especially erythrocyte ghosts, failure to find ferritin in nuclei of many of freed parasites and lack of ferritin attached to an occasional bacterium contaminating the preparation all suggested a degree of specificity. However, positive results obtained when ferritin alone (especially ferritin modified by conjugating agent) was applied suggested a nonspecific binding and stressed need to purify conjugates of unbound ferritin. Native ferritin was observed not only in cytoplasm of host cells but also within the parasites, particularly inside small single membrane-bound vesicles. (Supported in part by Agency for International Development, AID/csd-1432)

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Anti-lymphocyte Serum Effects on Plasmodium berghei Infection in Rats, DAN T. SPIRA and PAUL H. SILVERMAN*

The relative involvement of acquired cellular and humoral immunity in experimental malaria, and the connection between age resistance and immune mechanism were studied. Anti-lymphocyte serum (ATS) was prepared in rabbits against Lewis rat thymocytes. Lewis rats of two different ages (6 to 7 weeks, weighing about 130 grams and 9 to 10 weeks weighing about 166 grams) were treated with ATS, normal rabbit serum (NRS) or normal saline. One ml. of the respective serum was injected IP on days -1, 1 and 3 relative to infection. The serums were absorbed with normal rat erythrocytes to avoid blood loss due to anti-erythrocyte contamination. The parasitemia in ATS-treated rats of both age groups infected with 4 x 10^6 P. berghei NK65 parasitized rat RBC was greatly enhanced. The infections were fulminating and resulted in deaths of 14/15 rats. Of the 16 NRS or saline-treated young rats, 7 died, the others recovered after high parasitemia. The older rats treated with NRS or saline experienced a low transient parasitemia. The results indicate that lymphocyte mediated humoral antibody is a crucial factor in malaria immunity. Furthermore, that same mechanism seems to govern the age resistance in P. berghei-infected rats. (Supported in part by Agency for International Development, Contract AID/csd-1432.)

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Effect of immunosuppression on recovery from primary P. berghei infection of mice. LEE R. BARKER* and KENDALL G. POWERS

The effect of two immunosuppressive agents, anti-lymphocyte serum (ALS) and hydrocortisone, upon recovery from rodent malaria has been studied. The 17-X strain of Plasmodium berghei, which was used, causes a self-limited infection in mice.

Groups of mice received either hydrocortisone, ALS, or normal rabbit serum (NRS) by the intraperitoneal route every three days throughout the infection, beginning six days prior to inoculation. Parasitemias were followed daily on thin blood films, and hematocrit and fluorescent antibody levels were determined weekly using tail blood.

ALS and hydrocortisone treated animals developed much higher peak parasitemias than untreated and NRS treated controls. Average peak values for ALS, cortisone, and control groups were 80%, 75%, and 35% respectively. All control animals had recovered by the end of three weeks while ALS and hydrocortisone animals recovered only after four to five weeks. Both immunosuppressed groups developed more severe anemias than their controls. Antibody to P. berghei was demonstrable by the second week in control animals but appeared only after four weeks in immunosuppressed animals.

We conclude that mice treated with either ALS or hydrocortisone are able to recover from P. berghei infections, but they undergo a more severe infection and recover later than untreated controls. The appearance of specific antibody coincides with clearance of parasitemias in all animals, suggesting that humoral immunity is essential to recovery from a primary infection.

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Plasmodium vivax Infections in Ateles Monkeys, J. A. PORTER and M. D. YOUNG*

Ateles fusciceps were infected with Plasmodium vivax from Aotus trivirgatus hosts and from other Ateles. Successes were greater from the latter hosts. The infection has been through several serial passages. The average periods were: prepatent, 13 days; patent, 25 days. Maximum parasitemias were 23,890/cmm. Ateles geoffroyi was infected from A. fusciceps hosts. The average periods were: prepatent, 8 days; patent, 36. Maximum parasitemias were 24,350/cmm. Attempts to infect Ateles with P. vivax from human sources failed.

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Serological Relationships of Plasmodium Species in Malaria IFA Tests, A. J. SULZER* and MARIANNA WILSON

Morphological similarities of primate malarias and, more recently, serological studies have led to conclusions by several workers of certain close phylogenetic relationships. When we serologically compared Plasmodium falciparum and P. reichenowi which are morphologically almost identical, we could not, at first, find any difference. Only after designing a test procedure whereby minute variations in results could be reliably detected could we find serological differences.

By careful quantitation of variables, we found that reactions of appropriate antisera to the two species differed, on the average, by less than one twofold dilution, the heterologous system giving the lower reaction. A similar situation exists with P. brasilianum and P. malariae. The differences are much less than, for instance, differences between reactions of P. falciparum and P. vivax. The method, which includes a means of quantitatively showing minute differences in serological reactions, furnishes a tool for study of phylogenetic relationships and a means of serotyping Plasmodium.

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Mosquitoes Feeding on Engorged Mosquitoes. A. B. WEATHERSBY*, HYONG-SUN AH and JOHN W. McCALL

Aedes aegypti (L.) have been observed feeding on freshly engorged mosquitoes in small cages. The unfed mosquitoes were attracted to and fed avidly on the host mosquitoes which offered little resistance other than movements that were noted when mosquitoes touch each other. Feeding in this manner was more vigorous soon after engorgement of the donor mosquitoes but was observed as late as three hours after engorgement. Blood stages of Plasmodium gallinaceum Brumpt were recovered from mid-guts of the thieving mosquitoes. The confined environment of the small cages afforded opportunities for sub-feeding that might not be present in nature.

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Immunologic Studies on Larval Echinostoma lindoense in the Snail Host, D. HEYNEMAN* and W. P. FAULK

The parameters of an immune system have been developed for E. lindoense in order to study snail response to trematode infection and to follow antigenic correlates of Echinostoma development. Rediae, washed free from snail tissues, were solubilized by ultra-sonication and insoluble debris was discarded after high-speed centrifugation. Molecular heterogeneity of the antigen was studied in cellulose acetate and on Sephadex G-200. Electrophoresis revealed 2 bands, but molecular sieve chromatography demonstrated 3 populations: macromolecular, intermediate, and micromolecular. The heterogeneous antigen in Freund's complete adjuvant was inoculated intramuscularly weekly for 6 weeks using 1 mg of antigen protein. Failure of a rabbit response was followed by an injection-free period of 1 month, then by simultaneous injection of 1.25 mg alum-precipitated antigen in the muscle mass of each limb. Antiserum harvested in 12 days contained precipitating antibodies against whole antigen, micromolecular antigen protein, and snail ovotestis. Anti-snail immunoglobulins were absorbed out with soluble ovotestis protein, and resultant immune complexes were removed by ion-exchange chromatography over DEAE-cellulose. The immunochemically pure IgG was quantitated for specific antibody with hemagglutination. Heat inactivated antigen was coupled to indicator erythrocytes via chromic chloride. The antiserum gave an anti-larval titer in excess of 1:1024.

(Supported in part by USPHS NIH Grant AI07054.)

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Immunological Studies on the Canine Hookworm, Ancylostoma caninum, J. C. WILLIAMS

Antigenic components of A. caninum infective larvae were enumerated and characterized by immunoelectrophoretic analysis. Rabbits were immunized with soluble extract and particulate homogenate antigens in association with Freund's complete and incomplete adjuvant. Sera derived from immunization with homogenate antigen and incomplete adjuvant showed less precipitin reactivity than other sera evaluated. The larval antigen was separated into 18 distinct components and an adult A. caninum antigen yielded 13 components. Reaction of larval and adult antigens with respective heterologous immune sera indicated 12 cross-reacting systems. A Necator americanus adult extract antigen formed 5 bands with anti-larval A. caninum immune sera and 4 with anti-adult A. caninum sera. Sera from patients infected with N. americanus formed 4 bands with the homologous antigen and 1 band each with the A. caninum antigens. A bacterial contaminant (Proteus sp.) of the A. caninum antigens was shown to be unrelated to the larval A. caninum antigen. Treatment of the larval antigen at various pH levels (4.0, 6.0, 10.0, and 12.0) resulted in denaturation of some components. Larval antigenic components were non-dialyzable against the stock buffer system. The larval A. caninum antigen was largely protein in composition and extremely heat labile at 100 C for 1 hr and under autoclave conditions; the polysaccharide nature of 2 heat stable components was suggested. Freezing and thawing had no effect on the larval antigen. The larval and adult A. caninum antigens were separated into 7 protein zones by thin-layer chromatography on silica gel. (Supported by N.I.H. Grant 5-F1-GM-29, 183-03.)

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Some Biochemical aspects of the

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Systematics of Proterometra spp. (Trematoda:Azygiidae),
W.B. LUSHBAUGH* and M.G. ANDERSON.

Paper chromatography of ethanol extracts (developed with ninhydrin) and gas chromatography of esterified methanol extracts of six species of Proterometra was undertaken. The results gave chemical confirmation of the identity of each of the species of Proterometra studied. Comparisons of the chromatographically separated compounds were used to establish indices of affinity by which degrees of relationship between the species were inferred. Close agreement of the indices derived from each method of analysis and that presumed from conventional criteria appears to be significant. Four subgeneric groups were indicated. Group I contained P. dickermani, P. saggittaria and P. albacuada; Group II contained P. macrostoma; Group III, P. septimae and P. catenaria; and Group IV, P. hogesiana. The possible pattern of evolution in these species will be presented in the form of a dendrogram.

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Changes in the Free Amino Acid Concentration of Bile During Infection by Fasciola hepatica L.
HADAR ISSEROFF* and CLARK P. READ.

An amino acid analysis of bile from the biliary ducts of rats, rabbits and cattle was made. The free amino acid concentration was found to be significantly higher in animals harboring mature Fasciola hepatica. Total amino acid concentration in infected bile increased from two to ten times. The increase in proline concentration, however, was much more marked. Changes in amino acid concentration of bile with progressive infection were followed in the rat. Proline concentration increased about 10 fold while the young worms were still in the liver parenchyma, but increased to over 1000 fold after the worms entered the bile ducts. The reasons for such increases in amino acid concentration are not clear, however, in the case of proline, at least, it is suggested that the amino acid may be excreted as a nitrogenous waste product of the worm, as such a route might be metabolically expedient for the parasite.
(Supported in part by the USPHS through Grant AI-01384 and Postdoctoral Fellowship 5-F2-GM-21, 223-02.)

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Glucose Uptake and Glycogen Synthesis in Schistosoma mansoni
S. H. ROGERS* and E. BUEDING

Previous studies have demonstrated that Schistosoma mansoni has a high rate of glucose utilization; it is likely that at least some of this glucose is stored as glycogen in many areas of the parasite's body. This investigation is concerned with the demonstration of the anatomical localization of glucose uptake, and the histochemical visualization and biochemical characterization of uridine diphosphate glucose(UDPG)-glycogen transferase. Results obtained with an apparatus designed to determine whether glucose is absorbed via the digestive tract or via the cuticle confirm the theory that the primary area of glucose absorption in S. mansoni is the cuticle. Modifications of Sasse's histochemical technique permitted localization of UDPG-glycogen transferase. S. mansoni UDPG-glycogen transferase can exist in two forms. One form requires high concentrations of glucose-6-phosphate(G6P) in the medium before significant activity is demonstrable. Activity of the other form of the enzyme increases progressively as the concentration of G6P in the medium increases. The enzyme can be converted completely into either of its two forms, isolated, and analyzed. By incubation of whole worm homogenates at high and low G6P concentrations, the activity of each form of the enzyme can be determined. Possible relationships between glucose absorption and glycogen synthesis will be discussed.
(Supported by NIH grants 5-T01-AI-149 and 1-R22-AI-08022).

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Sequential Appearance of 3 Major Perivitelline-Fluid Macromolecules During Development of Ascaris lumbricoides var. suum,
D.B. SMITH

Ouchterlony analysis has provided evidence that 3 major proteins were synthesized or made soluble as the egg developed at 30°C. The macromolecules appeared sequentially during the following 3 periods of time: 0-5 days (morula), 5-10 days (vermiform), and 10-17 days (early infective). The precipitin bands representing the 3 macromolecules were also identified as the major proteins of the perivitelline fluid of the infective egg. Immunoelectrophoretic analysis of infective-egg, perivitelline fluid produced 6 major components, 4 of which formed 2 sets of identity reactions between fast and slow components. These findings may relate to vitelline-membrane permeability or nitrogen balance in a closed system.
(Supported in part by grant No. AI-04953 and Training Grant No. 5 TOI AI-226.)

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Ultrastructure of the Conoid Apparatus of Dormant and Activated Sporozoites of Eimeria,
JOHN M. VETTERLING* and P. A. MADDEN

The conoidal apparatus, which was first described by Gustafson, Agar and Cramer (1954) for Toxoplasma, has been reported in motile stages of many Sporozoa. It has been described in some detail by

several authors, but only a few have speculated as to its function. Garnham (1963) stated that it probably is a structural support for the anterior end of the organism. However, Ludvík (1963) hypothesized its function as a penetrating organelle. In investigations into the changes in sporozoites of poultry coccidia during excystation and penetration, we observed changes in the position of the conoid. Since many ultrastructural variations can be created by hyper- or hypontonic fixatives, we designed our fixatives based on the media in which sporozoites survived for extended periods of time. Sporozoite viability was evaluated by inoculating cell cultures with sporozoites from the same suspension as those that were fixed for electron microscopy. With this procedure we (1) avoided protrusion of the conoid due to osmotic pressure or alterations during morbidity, and (2) were able to determine ultrastructural changes when dormant sporozoites were activated and subsequently penetrated cultured cells. As reported by others, the conoid in dormant sporozoites was within or slightly posterior to the apical ring. However, after activation the conoid protruded anteriad to the apical ring. This feature, which was made possible by using a scanning electron microscope, was observed in nearly all activated sporozoites. After entering a cell, the conoid was retracted. Consequently, it is believed that the conoidal apparatus is used by sporozoites to penetrate cells.

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Habitat Segregation in Blood Flukes of Rockfishes from the Pacific Coast. JOHN C. HOLMES

Two species of blood flukes, *Aporocotyle* n. sp. and *Psettarium* n. sp., inhabit the heart and/or afferent branchial circulation of various species of rockfishes (genus *Sebastodes*) taken in the Puget Sound-Vancouver Island region. The habitats of the two species appear to be segregated by morphological, behavioural and ecological factors. *Aporocotyle* sp. is wider, has groups of spines along the margins of the body, and appears to be swept downstream in the branchial circulation until the spines catch. It is usually found in the afferent branchial arteries, less frequently in the ventral aorta, and rarely in the bulbus arteriosus or ventricle. *Psettarium* sp. is more elongate and is usually at least partially embedded in the musculature of the ventricle or in the fine branches of the atrium. *Aporocotyle* sp. appears to be limited to rockfish from the shallower (less than 100 fathoms) waters of Puget Sound or the Straits of Georgia and is most abundant in rockfish associated with shallow reefs. *Psettarium* sp. may be found in the same fish, but is more abundant in rockfish from non-reefy, deeper, waters of the continental shelf west of Vancouver Island.

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Pre- and Postimpoundment Surveys of Helminth and Crustacean Parasites of Black Basses, DAVID A. BECKER*

The effects of impoundment on the qualitative and quantitative fluctuations of helminth and crustacean parasites of the black basses of Beaver Reservoir in northwestern Arkansas were determined by comparative pre- and postimpoundment surveys over a period of 5 years. A total of 1,399 basses were collected by electrofishing and dissected for parasites which were prepared for identification using conventional methods. Parasites with direct life cycles (monogenetic trematodes, leeches, and certain copepods) found favorable impoundment conditions for increased per cent infection. In contrast, parasites with intermediate hosts in their life cycles (digenetic trematodes, cestodes, acanthocephalans, and nematodes) found impoundment conditions unfavorable due to separations from their intermediate hosts which evidently take a longer period of time to adjust to the rising water level and other unique environmental conditions encountered in a new reservoir. Subsequent investigations reveal that these adjustments in the life cycles are still taking place during eutrophication. (Supported by USDI, FWS, BSFW 14-16-0008-626 and -759, and OWRR through Arkansas WRRC A-009-ARK as authorized by the Water Resources Research Act of 1964, P. L. 88-379.)

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Preliminary Report on Some Trematodes of Fishes from South America. ERNEST J. HUGGHINS, South Dakota State University, Brookings, S. Dak.

A study of trematodes of fishes in the Amazonian regions is being undertaken with a view toward elucidation of phyletic relationships between fishes of South America and Africa. The ultimate objective is to discover further evidence for the theory of continental drift. The digenetic trematodes known from freshwater fishes of South America have come primarily from Brazil, Venezuela and Argentina. Virtually none have been reported from the upper Amazonian drainage system. In 1968, I collected some trematodes of fishes in Peru and Colombia. Amphistomes were found most frequently, which bears out Dr. H. W. Manter's observation that this is the predominant type of trematode in the lower Amazon. Manter considers it significant that amphistomes occur also in fishes of Africa and India but are practically absent in freshwater fishes elsewhere. Numerous specimens of a new species of Pseudocladorchis Daday, 1907 (Paramphistomidae), were taken from 3 kinds of catfishes ranging from the Ucayali River in east-central Peru to the Amazon at Leticia, Colombia. A second kind of amphistome, apparently a new genus, was found in a characid from the Pachitea River, Peru. Additional findings of particular interest were as follows: Electric eels from the Amazon near Leticia harbored a new species of Prionosoma Dietz, 1909 (Echinostomidae), a genus previously reported only from birds. Moving from the jungle to Lake Titicaca on the altiplano, the primitive catfish there, Trichomycterus rivulatus, showed a high incidence of infection with Phyllodistomum sp., but no other trematodes were found. Acknowledgment is

due H. W. Manter and Mary H. Pritchard for aid in identification. (Supported by NSF Grant GB-7067).

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Trematodes of the Genus Renicola Cohn, 1904 (Trematoda:Digenea:Renicolidae), E. E. Byrd,* R. W. Heard and F. E. Kellogg.

During 1966-1967 at least 3 species of flukes belonging to the genus Renicola were found in the kidney of birds from the southeastern section of the United States. Two of them came from the Clapper Rail, Rallus longirostris subspp., taken from Alabama and Florida. One of these was identified as R. hydranassae Lumsden and Zischke, 1963, taken from the Louisiana Heron, Hydranassa tricolor, in Louisiana. It was present in 3 of 23 rails from Pinellas and Monroe Counties, Florida. The record extend both the range of hosts and the species geographical distribution. The second rail form, considered to be new to science, came from 1 of 10 rails in Alabama and 16 of 33 rails in Pinellas, Monroe and Indian River Counties, Florida. The form appears to be distinct in that the two testes are fused, with each lobe giving rise to a vas efferens. The third member of the genus came from the wild turkey, Meleagris gallopavo silvestris Vieillot, taken from Tunica County, Mississippi. The fluke was present in 7 of 20 adult birds and their number ranged from 4 to over 200 per host. None was found in poults under 12 weeks of age. No other bird from the area was searched. The turkey form possesses three distinct testes. The new species are described elsewhere. (Supported by Southeastern Cooperative Wildlife Disease Study, School of Veterinary Medicine, University of Georgia, through funds made available by the Congress of the United States and administered through the Bureau of Sport Fisheries and Wildlife of the Department of the Interior, Contract Nos. 14-16-0008-676 and 14-16-0008-702 and the General Research Budget, University of Georgia).

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A New Record of Turtle Cestode in Louisiana.
A. D. ACHOLONU

The literature on turtle cestodes is very meager. To date only two species of these parasites have been described from turtles in the United States, viz., Proteocephalus trionychinum (Lönnberg 1894) and P. testudo (Magath. 1924), and both from softshell turtles. McKnight 1959 (Dissert. Abstr 20 (3): 1106) reported recovering Cylindrotaenia americana, a parasite of amphibians from Trionyx spinifer and T. ferox emoryi.
A total of 180 turtles comprising 12 different species collected from southeastern Louisiana were autopsied between the springs of 1965 and 1969. Only one species, T. spinifer was found to be infected with Proteocephalus sp., probably P. trionychinum. Of 18 of these collected from False River, New Roads, 50% were infected with worms ranging from one to 35 in number.
This is the first report on the incidence of turtle cestode in Louisiana.
(Supported by Institutional Grant No. 8-1281)

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Wyominia tetoni: Its Hosts, Distribution, and Other Relationships, REX W. ALLEN

Wyominia tetoni Scott, 1941 is an anoplocephalid tapeworm described originally from bighorn sheep (Ovis canadensis canadensis) in Wyoming. It has never been reported from a host other than a bighorn or mountain sheep. Subsequent to the original finding the tapeworm was recorded from O. c. mexicana and O. c. nelsoni which are desert varieties of mountain sheep, and from O. dalli which is the white mountain sheep of western Canada and Alaska. Wyominia tetoni grossly resembles Thysanosoma actinioides, another anoplocephalid tapeworm of ruminants. On more than one occasion, the two cestodes have been mistakenly identified as the same parasite, probably because both are about the same size, have an affinity for the biliary system of the host, and frequently are associated in concurrent infections. Both are New World parasites and in the United States have a western distribution. Unlike W. tetoni, T. actinioides parasitizes a wide range of ruminant hosts including the domestic sheep. Morphologic and biologic relationships between the two cestodes will be discussed.

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Ecology of the Acanthocephalan, Acanthocephalus dirus, in the Intermediate Host, Asellus intermedius (Crustacea: Isopoda). ARTHUR J. SEIDENBERG

A population of Asellus intermedius, a fresh-water isopod, serves as an intermediate host of the acanthocephalan, Acanthocephalus dirus, in a small stream in east-central Illinois. The isopod population was qualitatively sampled for 20 months, from January, 1967, to August, 1968, to determine the life cycle of the parasite in the intermediate host. Isopods acquire the worm larvae in the summer. The incidence of infection rapidly increases through the fall and winter months, reaching its highest values in March and April. In October and December, 1967, 21% and 26%, respectively, of the larvae present were acanthellae. By March and April, only 3% and 5%, respectively, of the larvae were still acanthellae. There is no difference in susceptibility to infection between male and female isopods. However, there is a distinct difference in the pattern of infectivity. The relationship between size and incidence of infection is linear for female isopods. The larger a female is, the more likely it will be infected, while this relationship holds true for male isopods only up to about 10 mm in length. Above 10 mm in length, the per cent of male isopods infected levels off to about 40%. On each of four sampling

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dates, female isopods carried more worm larvae than male isopods, although the differences were not statistically significant. Early in the life cycle of the parasite, in October, isopods carry significantly more larvae than later that winter or in the following spring. (Supported in part by U. S. Department of the Interior Grant A-018-ILL).

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Ectoparasite-Host Relationships among Pichindé (Colombia) Small Mammals.

HAROLD TRAPIDO+

It is uncertain whether Pichindé virus, which is serologically related to the etiological agents of Argentinian and Bolivian haemorrhagic fevers, and has been repeatedly isolated from Oryzomys albigularis, is transmitted by arthropods. In seeking evidence on this point, detailed collections of ectoparasites have been made during the past several years from O. albigularis and associated small mammals. Ectoparasite groups recovered include laelaptine mites (ca. 12 species), trombiculid mites (ca. 17 species), ixodid ticks (2 species), Siphonaptera (8 species), Anoplura (rare) and staphylinid beetles of the tribe Amblyopinini (4 species). One ectoparasite species, Gigantolaelaps inca, has been found to be highly host specific on O. albigularis and Pichindé virus has been recovered from it, but in circumstances which do not preclude possible contamination by host blood or tissue fluid in the arthropod gut. (Supported in part by the Universidad del Valle, The Rockefeller Foundation and AFSOR Grant 68-1558.)

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Amblyomma (Acarina:Ixodidae) on white-tailed deer from south Texas.

W. M. SAMUEL.

A study was conducted during 1966-1968 to determine the tick fauna on white-tailed deer from the Welder Refuge, south Texas. The prevalence and abundance of several species of Amblyomma were compared with 1) host age, sex, and behavior, 2) weather, and 3) landscape density and soil type. A total of 143 neonatal fawns and 261 older deer were examined for ticks. Three species (Amblyomma americanum, A. inornatum, and A. maculatum) were successful parasites on Welder deer. Adult A. americanum were not found on neonatal fawns, adult A. inornatum preferred this aged host, while A. maculatum was found on deer of all ages. Larval and nymphal Amblyomma attacked several fawns hours after birth. A. maculatum preferred to attach to the developing antlers of males each summer. Conditions for ectoparasite development and activity were apparently most favorable in dense vegetation habitats. The potential of Amblyomma's to transmit Theileria infections, a common infection of Welder deer, is discussed.

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